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LIVESTOCK PARASITOLOGY IN THE UNITED STATES*

BENJAMIN SCHWARTZ
Bureau of Animal Industry, United States Department of Agriculture

Although the Act of the Congress of the United States establishing the Department of Agriculture became effective in 1862, twenty-one years elapsed before animal disease investigations on a sustained basis were undertaken by the Department. In 1883 a Veterinary Division was established in the Department of Agriculture. In the next year the Congress established the Bureau of Animal Industry in the place of the Veterinary Division, and Daniel E. Salmon, a capable young veterinarian who had made investigations for the Department of Agriculture and later headed its Veterinary Division, became the chief of the new Bureau. The Bureau of Animal Industry, besides being given other responsibilities, was charged with the duty "to provide means for the suppression and extirpation of contagious pleuro-pneumonia and other contagious diseases among domestic animals."

Even before the Bureau of Animal Industry came into existence, the livestock and meat industries of the United States had already developed to such an extent that they had sizable surpluses for export. Beef and pork, as well as live animals, had been exported for a number of years to various European countries. Unfortunately, several countries had placed restrictions against these importations on account of disease which might be conveyed to their native stock, or because of human health hazard in consuming meat from diseased animals. Aside from the export restrictions, American stockmen faced serious difficulties from the diseases to which their stock at home was subject. Among these diseases were some of parasitic origin, the nature of which either was not understood, or for which effective control measures had not yet been developed, or put into general use.

The disease which was then known as murrain, or splenic fever, or Texas fever, caused great consternation among livestock producers, especially in the northern States, who saw their animals stricken and die when they followed the trails of apparently healthy cattle that had been driven northward from Texas and elsewhere in the South. So great was the fear among northern cattle owners of the danger to their herds from southern cattle, that rigorous action was taken, sometimes accompanied by threats of violence, to prevent cattle from Texas from being driven over the trails which led to the northern markets. Another parasitic disease, known as sheep scabies, was ravishing flocks on the western ranges. On account of the highly contagious nature of this skin disease, the importation of sheep from the United States to other countries was barred, and in this country the disease was spreading as a result of the uncontrolled movement of livestock. Cattle scabies,

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too, lurked in the background as a disease requiring serious attention, if the increasing herds were to be maintained on a high level of efficiency. The internal parasites of the country's livestock were practically unknown at the time the Bureau of Animal Industry was established. However, one parasite of swine, *Trichinella spiralis,* was well known in this country, and abroad it was regarded as exceedingly dangerous on account of its potential harmful effects on human beings. By 1881 several countries in Europe had already placed an embargo on pork from this country, and the Department of State instituted, therefore, an inquiry to ascertain the causes that might render this meat dangerous to human health.

These, then, were the principal known problems in parasitology in relation to an expanding livestock production that confronted the Bureau of Animal Industry in 1884, when Salmon and his few assistants began "to provide the means for the suppression and extirpation" of diseases of farm animals. The manner in which the Bureau fulfilled its responsibilities, the investigational work that had to be carried out before suppression and extirpation could be undertaken, the means adopted to carry out the programs, and the success achieved, constitute an important and fascinating chapter in the history of livestock hygiene and disease prevention. Only those parts of the chapter which relate to parasites and parasitic diseases can be reviewed in the course of this address, and only by a few outstanding examples.

Tick Fever

Although it is uncertain when the disease now known as tick fever, or bovine piroplasmosis, was first introduced into the American colonies, it is probable that it came in with importations of cattle from the Spanish West Indies and Mexico, perhaps sometime in the 17th century. By the end of the 18th century the disease was apparently well entrenched in the United States, because by 1795 the State legislature of North Carolina prohibited by law the driving of any cattle from the low, coastal areas to the highland parts of that State, between the first day of April and the first day of November. In 1814 Virginia barred cattle from certain parts of South Carolina, and in 1836 North Carolina barred cattle from South Carolina and Georgia, between the dates aforementioned. Despite these and similar restrictions, tick fever continued to spread with the growth of the livestock industry and the development of transportation facilities and markets. Gradually it spread over the entire South, and often invaded different sections of the North. In 1867 the Kansas legislature appealed to Congress to authorize the Department of Agriculture to investigate Texas fever. By 1877 the disease had caused much harm throughout the country and continued to spread, despite the energetic methods that were being adopted to stem its tide. When the overland movement of cattle proved difficult or illegal, animals were moved by boat and landed in Northern markets, with the result that their contact with Northern cattle decimated prize herds that were being established after the Civil War.

An investigation was made in 1868, partly at the instigation of stockmen and partly under Federal auspices, by Professor John Gamgee, a British expert on animal diseases, assisted by two medical officers of the U. S. Army and others. Among the conclusions reached were that cattle "become affected in consequence of the nature of the soil and vegetation on which they are fed, and the water which they drink." With regard to the popular idea then prevalent that ticks had

something to do with the disease, Gamgee stated "that there is not the slightest foundation for the view that ticks disseminate the disease." In another place Gamgee stated, "The tick theory has acquired renown during the past summer but little thought should have satisfied anyone of the absurdity of the idea." Salmon, who in 1879 investigated tick fever under a commission from the Department of Agriculture, reported in 1880 as follows: "The tick theory scarcely explains a single one of the many peculiar phenomena of the disease," a conclusion which was generally shared at the time by those best informed on animal diseases.

Following the organization of the Bureau of Animal Industry in 1884, and the opening at the same time of an Experiment Station on the outskirts of Washington, D. C., as part of that Bureau, an opportunity was afforded to the handful of young researchers whom Salmon had collected, to investigate the most pressing livestock maladies that were exacting a severe toll from the resources of farmers and stockmen. In 1888 studies on tick fever were already in progress. In that year Theobald Smith, the leading investigator of Salmon's small coterie of scientists, observed the destruction of the red blood corpuscles of cattle sick with tick fever. In the following year three important discoveries were made in the Bureau of Animal Industry with reference to ticks and tick fever as follows: (1) Smith observed a parasite in the red blood cells of the affected animals and, on the basis of this discovery, explained the breakdown of the erythrocytes and the various lesions he observed while conducting postmortem examinations of cattle that had succumbed to the disease. (2) While Smith was carrying out these studies, Kilbourne, who was in charge of the modest Experiment Station aforementioned, started an experiment which ultimately led to the elucidation of the mode of transmission of Texas fever. What Kilbourne did in 1889 is contained in the following statement, written by Smith as part of his contribubtion to the 6th and 7th Annual Reports of the Chief of the Bureau of Animal Industry for the years 1889 and 1890: "During the summer of 1889 Dr. F. L. Kilbourne in arranging the various enclosures at the Experiment Station for the exposure of native cattle to the infection of Texas fever, conceived the happy idea of testing the popular theory of the relation of ticks to the disease. This he did by placing southern (North Carolina) cattle with native cattle in the same enclosures and picking the ticks from the southern cattle as soon as they had grown large enough to be detected on the skin. This prevented any ticks from maturing and infecting the pasture with the eggs, and hence prevented any ticks from infesting native cattle subsequently. At the same time in another enclosure the ticks were left on the southern cattle. The natives in the latter field died of Texas fever; those in the former did not show any signs of the disease." (3) Curtice worked out the life cycle of the fever tick for which he created a new genus, naming it *Boophilus,* and determined for the first time, in connection with tick life-history studies, that this parasite spent its entire developmental cycle, beginning with the seed tick, on the same host, instead of dropping off the host after each molt. This important discovery made it possible to apply certain measures to control these ticks, and paved the way for tick eradication, which finally resulted in the extermination of bovine piroplasmosis from the United States.

Additional investigations, carried out jointly by Smith and Kilbourne between 1890 and 1892, established conclusively that neither the soil from pastures on which ticky cattle had grazed, nor direct physical contact of healthy with affected animals,

was involved in the transfer of the disease from parasitized to susceptible cattle. Moreover, by placing on susceptible cattle the seed ticks that had hatched from the eggs deposited by gravid female ticks, the symptoms and lesions of tick fever, as well as the causative microparasites, were observed in the animals which became experimentally infected. Finally, it was shown by blood inoculations that the microparasites, named *Piroplasma bigemima,* were the causative agents of the disease, and that the tick, *Boophilus annulatus,* merely served to transmit them.

The determination of the nature of the causative agent of tick fever and its mode of transmission, as well as the demonstration of the main facts in the life cycle of the arthropod carrier, is certainly among the outstanding achievements in microbiology. These discoveries, significant as they were from a scientific standpoint, did not by themselves ease the burden on livestock producers in the South, whose cattle could not be shipped to northern markets, except for slaughter, between the middle of February and the middle of November, in conformity with measures that had been adopted in the meantime to confine tick fever to the areas where it was enzootic. All the southern States, from Virginia to Florida, and extending westward to include Texas and Oklahoma, were in the enzootic area. These States, along with California, were sooner or later placed under Federal quarantine. Therefore, to meet the practical needs of cattle producers, and to afford relief to a large section of the country which was seriously hampered in the development of a well-rounded agricultural economy, it became necessary to devise means for freeing cattle in the South and elsewhere of fever ticks. Investigations were begun in 1892 in which many veterinarians outside the Bureau of Animal Industry participated. These investigations were continued for several years beyond the initiation, in 1906, of a systematic campaign, under joint Federal and State auspices, with the objective of eradicating cattle fever ticks from the United States.

During a period of about 15 years various materials were tested as smears, sprays, and dips for the destruction of cattle ticks, in an effort to find a chemical that would destroy these arthropods without injuring their hosts. Substance after substance, as well as various combinations of substances, were tested and discarded, because the ticks proved to resist them much better than the cattle. As a desperate measure, cattle were even driven into the ocean's surf, in the hope that the pounding of the waves would dislodge the ticks. By 1903 crude petroleum was generally accepted as superior to any other tickicide discovered up to that time. Other control measures were tried, including active and passive immunization of cattle against piroplasmosis; starving the ticks by removing the cattle from the pastures contaminated with tick eggs and larvae, and keeping the pastures vacant for several months; and rotation of cattle on pastures, based on the demonstrated facts in the life history of the arthropod vector. These and other measures were tried as adjuncts to, or substitutes for, dipping of cattle in medicated baths.

In 1906 experiments were initiated with arsenical dips, which had already been used in Australia and Cuba for tick eradication. Arsenic proved to be superior to any other tickicide that had been used up to that time in this country. By dipping tick-infested cattle in arsenical solutions every two weeks, which was the procedure finally adopted, most of the ticks on the cattle were poisoned by the chemical. Ransom determined that the engorged ticks which survived dipping laid fewer eggs

on the ground than those from untreated cattle. Of the larvae that hatched from the eggs laid by the dipped female ticks, many died before they could get onto cattle. The seed ticks which succeeded in crawling onto cattle and living there were destroyed by the next dipping in arsenic. In short, the cattle were used as the collectors of the seed ticks present on the pasture, and the arsenical solution was used to destroy them on the cattle. With successive dippings the numbers of ticks found on cattle maintained on tick-infested pastures gradually diminished and disappeared altogether after several months of repeated dippings, at intervals of two weeks, throughout the spring, summer, and early fall months.

I have dwelt at considerable length on tick fever because it was the first parasitic disease of livestock on which an all-out attack was made in this country, with a successful outcome, and also because the story of tick fever in the United States is unparalleled in the annals of disease control, human or animal, anywhere in the world. With no definite knowledge whatsoever available in the beginning as to the cause, or mode of transmission, of the disease, all the facts pertaining to its nature and mode of spread—which certainly was a surprising one at the time it was made—and habits and mode of life of its arthropod vector, were brought to light as a result of research caried out in one place. Moreover, the means whereby the vector could be destroyed, without unduly injuring the host, also were discovered. This paved the way for cutting the life line by means of which the piroplasms were carried from the blood of one bovine to another. Finally, on the basis of the facts ascertained through long-sustained experimentation, a death sentence was pronounced on the two arthropod carriers of the disease, *Boophilus annulatus* and *Boophilus microplus,* and on the sporozoan parasites which they conveyed to their bovine hosts. That this sentence was carried out after more than four decades of strenuous effort, and in the face of strong opposition, is certainly a tribute to the research workers who forged the weapons of tick destruction, and to the many who used them.

Cattle fever ticks and the disease they transmit have been eradicated from the United States, except from a long, narrow strip of territory adjacent to the Rio Grande River. This strip, which is about 500 miles long, extends from Devils River near Del Rio, Texas, to the Gulf of Mexico, and to an average depth of 4 to 5 miles. As far as can be foreseen now, the cattle in this buffer zone will remain under quarantine until fever ticks are eradicated from Mexico. Despite the systematic dipping of cattle in the buffer zone, the drifting of animals from both sides of the river maintains the tick infestation, accompanied by occasional cases of piroplasmosis.

Sheep Scabies

At no time did scab or scabies of food-producing animals present a challenge that was even remotely comparable to that presented by the once mysterious malady called Texas fever. In 1884 the cause of sheep scab was well known, as was also the life history of the mite, *Psoroptes equi* var. *ovis.* The research that brought these facts to light was done in the first half of the 19th century, when it was shown by experiments that scabies in sheep did not develop in the absence of mites, and could be produced experimentally by transplanting the specific scab mites from affected to healthy sheep. It had been established also that the losses from this dis-

ease were severe, and resulted from the shedding of the wool, marked emaciation, anemia, and exhaustion which finally ended in death of a large percentage of untreated animals.

Before the turn of the century sheep in large areas of this country were affected with scabies, and many stockmen were forced to foresake sheep raising on account of the ravages of this disease. In fact, so severe were the monetary losses sustained by owners of scabby flocks, that Salmon and Stiles in 1898 regarded this disease as second only to hog cholera, from the standpoint of the loss of invested capital in livestock raising. The large bands of sheep on the Great Plains and in the Rocky Mountain region, as well as sheep in the feeding centers farther east, were most severely affected. Moreover, diseased sheep from those areas were sent to the large markets of the country, thereby spreading scabies almost everywhere. As a consequence of the uncontrolled marketing of scabby sheep, the stockyards became contaminated and many animals that were purchased there were likely to develop the disease.

Various plans were tried to arrest the further spread of scabies and to devise means of eradicating it. In 1895 a decree was issued, pursuant to authority granted by the Congress, prohibiting scabby sheep from entering stockyards or any other places where animals are handled for interstate trade, or to enter into interstate trade, but no apparent progress was made in checking the disease. Subsequent orders required the cleaning and disinfection of boats, railroad cars and other vehicles which had been used for the transportation of scabby sheep; prohibited railroads and other transportation companies, and captains of steamboats, from receiving for transportation, or from transporting from one State to another, sheep affected with scabies; and required, moreover, that all sheep shipped from stockyards to other States for feeding purposes be dipped in some preparation that would kill the mites. These devices alone did not show sufficient promise, however, in accomplishing the desired results. Even stationing of inspectors at shipping points in western States and at public stockyards to supervise dipping was insufficient to make a significant dent in the extent of the disease, or to sharply curtail its dissemination. It was not until a Federal quarantine was placed on all the territory west of the eastern borders of North Dakota, South Dakota, Kansas, Oklahoma, and Texas—an area covering 1,700,000 square miles—that a promising plan for extirpating sheep scabies from the United States actually got under way.

Under the new plan, inspections of sheep for evidence of scabies were made systematically on the farm and range. Treatment by dipping, under governmental supervision, in medicated solutions of established efficacy was also required for all flocks that were affected with, or had been exposed to, scabies. This plan, initiated in 1905 and still in effect today to a limited extent, resulted in the eradication of scabies from sheep in areas of this country where it was once widespread, and in reducing it elsewhere.

In 1898, Salmon and Stiles in a publication on sheep scabies reviewed critically the dips then in use, reported their own experiments with dips, and settled on two, namely, nicotine and lime-sulphur. These two dips have been used successfully ever since 1905, in millions of dippings. During the past few years, the Zoological Division of the Bureau of Animal Industry developed a dip that, in many ways, is

superior to, and much simpler to use than, the two that received official sanction. The active ingredient of the new dip is one of the chlorinated hydrocarbon insecticides, hexachlorocyclohexane, generally referred to as benzene hexachloride, or BHC for short. This chemical has been standardized for scabies eradication, on the basis of its gamma isomer content, to provide a margin of safety that should meet most of the likely contingencies that are apt to arise. The new treatment is rapidly gaining the approval of sheep producers and livestock sanitary officials. Through its use, the relatively small residue of what was once the most debilitating disease of ovine stock can be eradicated, I believe, in much less time than with the old treatments.

Trichinae in Swine

When Joseph Leidy reported in 1846 to the Philadelphia Academy of Natural Sciences the occurrence of trichinae in the superficial part of the the extensor muscle of a hog, he inadvertently took the first step that resulted in placing a stigma on pork produced in this country—a stigma which has persisted for many decades. By 1881 restrictive measures against the importation of pork from the United States were promulgated by various governments of continental Europe—Italy, Austria, Germany and France following one another in rapid succession. In the year before this prohibition went into effect, 70 million pounds of pork from the United States had been exported to France, and 43 million pounds to Germany. For the next 10 years pork from the United States was shut out by governmental decree from nearly every market on the continent of Europe. To regain this export trade, there was inaugurated in 1892 a system of microscopic inspection of all pork intended for export. This inspection, which was terminated in 1906, was carried out only to meet the requirements of the import countries, some of which required a similar inspection under their own meat hygiene practices. Under current Federal meat inspection there is no provision for microscopic inspection of pork intended for any purpose whatsoever. The abandonment of microscopic inspection of pork for export resulted from reports, especially from Germany, that trichinae were found from time to time in pork that had been imported from the United States and certified as free of these parasites. This was not surprising, considering the fact that it was well known in countries that had had experience with this scheme of prophylaxis that the detection of trichinae by microscopic inspection is, at best, a hit-or-miss method. The parasites were not discovered, as a rule, in lightly infested carcasses and were overlooked at times even in those that harbored sizable infections.

Early studies made in this country before 1891 showed that only about 2 per cent of the hogs were infected with trichinae. The routine microscopic inspections of pork that were made over a period of years thereafter showed that in over 8,000,000 hogs from which muscle tissue was examined microscopically, live trichinae were found in only about 1.5 per cent. Studies conducted a decade or so ago by the Bureau of Animal Industry by the far more accurate digestion technique also showed an over-all infection rate of about 1.5 per cent. When critically analyzed, however, the newer figures actually showed that in the intervening years there occurred a sharp reduction in the prevalence of trichinae in swine, especially in those raised on the farm. In one series of examinations, involving both microscopic inspection and digestion, it was found that in only 21 per cent of the diaphragms in which

trichinae were discovered by the digestion technique, could these parasites be demonstrated by microscopic inspection. During the past 2 years, trichinae in very small numbers were found in our laboratory by the digestion technique in about 1 per cent of about 1,200 diaphragms from Corn-Belt hogs, but only negative results were obtained when samples from the same diaphragms were examined microscopically with painstaking care. Therefore, the 1.5 per cent trichinous hogs discovered by microscopic inspection several decades ago undoubtedly represented only part of the infected carcasses that were then present in this country.

Unlike the findings in farm-raised hogs, in which the extent and degree of infection with trichinae are apparently on the downgrade, those in garbage-fed hogs which, fortunately, constitute only a very small percentage of the total hog slaughter in this country, continue to show a high incidence and a comparatively high degree of infection. Recent studies in our laboratories showed that over 10 per cent of garbage-fed hogs from the eastern seaboard still harbored trichinae, and that the degree of infection was so high that about half of the infected samples were detected by careful microscopic inspection alone.

Under Federal meat inspection, parasites and the lesions they produce in edible portions of carcasses must be removed by trimming before the carcasses or affected parts are passed for human food. If the infection or associated lesions are so extensive, however, that trimming would be impossible, or impractical, the carcass or part is condemned. Since trichinous pork does not differ in appearance from noninfected pork, it follows that trichinous hogs may be passed for human food, under Federal and other meat inspection, almost every day. Fortunately, however, raw pork, as such, is seldom eaten in this country intentionally, and then only by persons having a capricious appetite, or who have become addicted to this habit because of national origin or association with homes where this unhygienic dietary custom prevails. Cooked trichinous pork presents no danger whatsoever, a fact of which Leidy was well aware when he explained to the Philadelphia Academy of Natural Sciences in 1866 the circumstances under which he first discovered trichinae in pork 20 years earlier. Actually, he found these parasites in a slice of cooked pork he was eating, and stated that he had already satisfied himself that such meat was safe, because parasites generally were destroyed by thorough cooking.

The Bureau of Animal Industry has repeatedly informed the public that raw, or inadequately cooked or cured, pork is dangerous. In the absence of any known system of inspection whereby trichinous pork could be tagged and eliminated from the channels of trade, the problem has been met in the only other way that is possible and practical. Through its meat inspection service, the Bureau of Animal Industry rigidly enforces a requirement that no ready-to-eat article of food shall contain any muscle tissue of pork, unless that meat has been refrigerated, or heated, or otherwise treated, in a manner that will insure the destruction of trichinae. The background for these requirements lies in extensive investigational work, carried out by Ransom and his associates and by others in the Bureau of Animal Industry. In 1913 Ransom determined, following exhaustive tests, that certain low temperatures, compatible with the practical requirements of the meat industry, destroyed the vitality of trichinae. Later investigations established the fact that the heating of pork to a temperature of not less than 58° C., as well as certain curing procedures, also

destroyed these nematode larvae. These findings, translated into action by Federal meat inspection, have given to the American people for several decades a protection from pork-containing products that otherwise would have been the most fertile sources of trichinosis.

Early Investigations of Internal Parasites

Aside from trichinae and a few other helminths of livestock, little was known at the time the Bureau of Animal Industry was established about the kinds of parasitic worms that occurred in our domestic animals, and even less was known about verminous diseases. Curtice, Stiles, Ward, and a few others contributed much that helped to lay a foundation upon which those who followed built a sizable structure of knowledge of the helminths and the diseases they cause in food-producing animals. In this address only the early work on the helminth parasites of livestock will be mentioned.

Even while sheep scabies was receiving preferred attention, it was recognized that the internal parasites of ovines could not be ignored. Stomach worms already had a reputation as being injurious parasites of sheep, and it was assumed, moreover, that there might be others that had the capacity of doing serious harm. It is not surprising, therefore, that Curtice, who began his studies in the Bureau of Animal Industry in 1886, should have embarked on a study of the parasites of sheep. That study resulted in the publication of a treatise on the subject, which contained much that was new and significant. Perhaps the outstanding contribution made by Curtice, while engaged in this study, was the discovery of the cause of nodular disease which, because of the resemblance of its lesions to those produced by the tubercle bacillus, had been considered as intestinal tuberculosis and studied, therefore, from a bacteriological standpoint. Curtice determined, however, that the nodules were caused by nematode larvae, which he recognized to be the developmental stages of mature worms localized in the large intestine, and named by him *Oesophagostomum columbianum*.

Stiles, who followed Curtice as the parasitologist of the Bureau of Animal Industry, brought to bear on his investigations a wide knowledge of zoology and parasitology, together with a strong bend toward preparing comprehensive studies and reviews of the morphology, classification, and taxonomy of parasites of all sorts, including arthropods. Independently and in collaboration with Hassall, he contributed extensively to our knowledge of the cestodes of ruminants and of related tapeworms of rabbits and hares, parasites of importance in the inspection of meats, nematode parasites of ruminants, and related problems in parasitology. Stiles also was one of the earliest workers to investigate verminous diseases of ruminants, which he found to be associated principally with stomach worms and, to a lesser extent, with other helminths.

Ransom, who succeeded Stiles in 1903, resumed the studies of ruminant parasites begun by Curtice 15 years earlier, limiting his investigations to the nematodes, but extending them to include the roundworms of all the domestic ruminants. With the painstaking precision which characterized his scientific work, Ransom showed that the nematode fauna of ruminants in this country was richer than his predecessors had recognized or suspected. He established, moreover, sound and concise

morphological criteria for the identification of the genera and species involved. In his classic study of the life history of the stomach worm, *Haemonchus contortus,* and of other nematodes of ruminants, he determined that there was a pattern of larval development and behavior, which has since been found to fit, in a general way, the strongylid nematodes of herbivorous animals as a whole. Ransom's investigations of ruminant parasites were brought together in a monograph on the nematodes parasitic in the alimentary tract of cattle, sheep, and other ruminants. Though this study was published in 1911, it is still a useful and prized possession of livestock parasitologists the world over, and in demand even today.

Investigations of Anthelmintics

Some years ago a pharmacologist in one of our leading medical schools called attention to the fact that most of the anthelmintics then known were derived from plants. He even speculated that this might indicate a fundamental antagonism between animals and plants, the one group being capable of producing substances that are more or less injurious to the other. That most of the older anthelmintics were of plant origin is evident from the mere enumeration of such substances as turpentine, areca nut, thymol, kamala, chenopodium, male fern and santonin, among others.

In 1918 Hall and Foster published the results of an experiment involving most of the then known veterinary anthelmintics, and concluded that many, which by tradition had been regarded as highly efficacious, were actually without merit. They discarded one after another of most of the older vermifuges they investigated, and gave a nod of approval to only a few, namely, copper sulphate, oil of chenopodium, oleoresin of male fern, turpentine, and nicotine. Shortly after the publication of these results, Hall introduced into the armamentarium of anthelmintics two synthetic substances, both chlorinated hydrocarbons, namely, carbon tetrachloride and tetrachlorethylene. Though these compounds were found to have some application in the treatment of livestock for the removal of helminths, their chief value lay in their efficacy for the removal of hookworms from man and carnivores. Several related synthetic compounds, notably normal butyl chloride, normal butylidene chloride, and hexachloroethane, also were found to be of value in medicating parasitized livestock.

Aside from carbon tetrachloride and hexachloroethane, which are still used in treating domestic ruminants for the removal of liver flukes, the chlorinated hydrocarbon anthelmintics, as well as most of the other anthelmintics used earlier, have been replaced to a great extent by more effective drugs. This is due principally to a discovery made in the Bureau of Animal Industry in 1939, concerning the anthelmintic efficacy of thiodiphenylamine, or phenothiazine. This substance was synthesized in 1885, but lay dormant for many decades before anyone thought of using it for therapeutic purposes. First brought into experimental use as an insecticide and later as a urinary antiseptic, it was found to be of great value in treating horses, cattle, sheep, goats, swine and poultry for the removal of certain nematodes. Though launched as an anthelmintic only two years before we became involved in World War II, 3 million pounds of phenothiazine were manufactured in the United States for anthelmintic use in 1944, to protect our livestock, especially sheep, from the depredations of roundworm parasites. Today, when our livestock must again

be carefully safeguarded as a defense measure, the annual production of phenothiazine in this country is about 5 million pounds, and would greatly exceed that figure if present-day shortages did not limit the volume of production. A number of foreign countries, especially those with large sheep populations, have been making strong appeals to this Government for allotments of phenothiazine or the parent substance, diphenylamine, adequate to protect their sheep and other livestock from parasites in times of emergency.

Because anthelmintics are more or less specific in their action, investigators working in this field are faced with the responsibility of discovering more effective drugs than are now available for the treatment of specific verminous diseases. Recently, sodium fluoride came into use as a treatment for the removal of ascarids from swine and has almost displaced all other treatments previously used for this purpose. Lead arsenate is rapidly displacing older treatments for the removal of tapeworms from ruminants. The recent discoveries of anthelmintic drugs also have opened up opportunities to parasitologists for careers with commercial firms that manufacture or formulate anthelmintic and other parasiticidal chemicals.

DISCUSSION

It is evident from the discussion of even a few of the problems with which the livestock parasitologist in the United States has been dealing, that the research findings and action programs based on them have been directed mainly to the conservation and increase of food and fiber, needed by a population that has been steadily increasing. However, it is evident also that the production of livestock cannot be increased indefinitely, because our available grasslands and our capacity to grow livestock feed are limited to a large extent by our geographic boundaries. Since extending our geographical frontiers is certainly not part of our national policy or ambition, our increased food production in the future will require, among other things, pushing steadily to the new frontiers that are opened up by scientific discovery.

How soon we shall reach a saturation point in our ability to support the increasing numbers of livestock that will be needed in keeping with the growth of our population, and how extensive this increase will have to be, cannot now be predicted. It should be borne in mind, however, that great progress is already being made in developing genetically superior strains of food-producing animals and in discovering superior methods of preventing virus, bacterial, parasitic, nutritional, and other diseases of animals, as well as suppressing their insect pests.

Whether we shall be able to support indefinitely an increasing population is a question that has already aroused considerable discussion. The neo-Malthusians, who take the pessimistic view, foresee dire consequences in increasing populations, especially in countries that are already overcrowded. They regard the introduction of new and improved public health measures into the so-called backward countries as merely hastening there the approach of mass starvation. Also, they charge that the introduction of life-prolonging measures merely aggravates the food problem in those parts of the world that already have teeming populations, now living on a low nutritional plane, if not actually facing famine. In fact, they ask bluntly what is accomplished by saving millions of people from malaria and other diseases in the

world's most congested areas, if this will merely result in giving them added time to suffer from malnutrition and finally die of starvation.

These are, indeed, difficult questions to answer today, in a world that is already beset by more perplexities than it can find time to resolve. The livestock parasitologist adds little or nothing to the world's perplexities, however, but like other agricultural scientists, offers much that can help to resolve them. The livestock parasitologist is a conservationist, being essentially concerned with maintaining and increasing the supply of human food which, in the final analysis, is the greatest of our natural resources.

NEW BLOOD FLUKES (TREMATODA: SPIRORCHIDAE) FROM THE MARINE TURTLE, *CHELONIA MYDAS* (L.)

W. E. Martin and J. W. Bamberger

Allan Hancock Foundation and Zoology Department
University of Southern California

INTRODUCTION

During one of the cruises of the University of Southern California's scientific vessel, Velero IV, under the command of Captain Allan Hancock, two marine turtles, *Chelonia mydas* (L.) were obtained on April 4, 1949, from the keeper of the Cape Lazaro Lighthouse, Baja, California. The lighthouse keeper collected them at the entrance to Santa Maria Bay. These turtles were examined for parasites and one was found to harbor two species of blood flukes in the mesenteric veins. Although these flukes are sufficiently similar to be placed in the same genus, they cannot be included in any existing genus. Therefore, the generic name *Haemoxenicon* is proposed for them. We wish to acknowledge the kind assistance of Dr. A. H. Weston, Professor of Classical Languages, University of Southern California, in the construction of this name.

Blood flukes from a marine (?) turtle were first reported by Leared (1862). He did not identify the host beyond calling it the "edible" turtle. However, Monticelli (1896) found what he believed was the same species, *Distoma constrictum* Leared, in the heart of a marine turtle, *Thalassochelys caretta* L. Because the name *Distoma constrictum* was preoccupied, Monticelli named this species *Mesogonimus constrictus* (Leared). What was believed to be the same species was reported by Looss (1899) from a marine turtle, *Thalassochelys corticata,* collected along the coast of Egypt. He erected the genus *Hapalotrema* for Leared's species. Price (1934) believed that Leared was dealing with one species but that Monticelli and Looss were dealing with another. Therefore, he proposed *Learedius europaeus* for *D. constrictum* Leared and used *Hapalotrema mistroides* for the species described by Monticelli and Looss. Additional species of blood flukes from marine turtles are: *Hapalotrema synorchis* Luhman, 1935, from the heart of *Caretta caretta,* the loggerhead turtle; *Neospirorchis pricei* Manter and Larson, 1950, and *Carettacola bipora* Manter and Larson, 1950, from *Caretta caretta; Learedius orientalis* Mehra, 1939, and *Monticellius indicum* Mehra, 1939, from *Chelonia mydas; Amphiorchis lateralis* Oguro, 1938, from *Eretmochelys squamosa* (Girard); *Learedius learedi* Price, 1934, and *L. similis* Price, 1934, reported from *Chelonia mydas* by Nigrelli (1940, 1941); and *Neospirorchis schistosomatoides* Price, 1934, *Amphiorchis amphiorchis* Price, 1934, *Learedius learedi* Price, 1934, and *L. similis* Price, 1934, from *Chelonia mydas.*

All turtle blood flukes thus far reported in the literature belong to the family Spirorchidae Stunkard, 1921, and these have been reviewed recently by Byrd (1939) and Mehra (1939, 1940).

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MATERIALS AND METHODS

The flukes were removed from the mesenteric blood vessels of the turtle and killed without pressure in cold Bouin's solution. Totomounts were stained with paracarmine and serial sections with hematoxylin and eosin. Permount was used as a mounting medium.

There was a marked size difference in the two species of flukes. Five of the larger size, four as whole mounts and one from serial sections, were studied. Twelve of the smaller type, eight as whole mounts and four from serial sections, were studied.

OBSERVATIONS

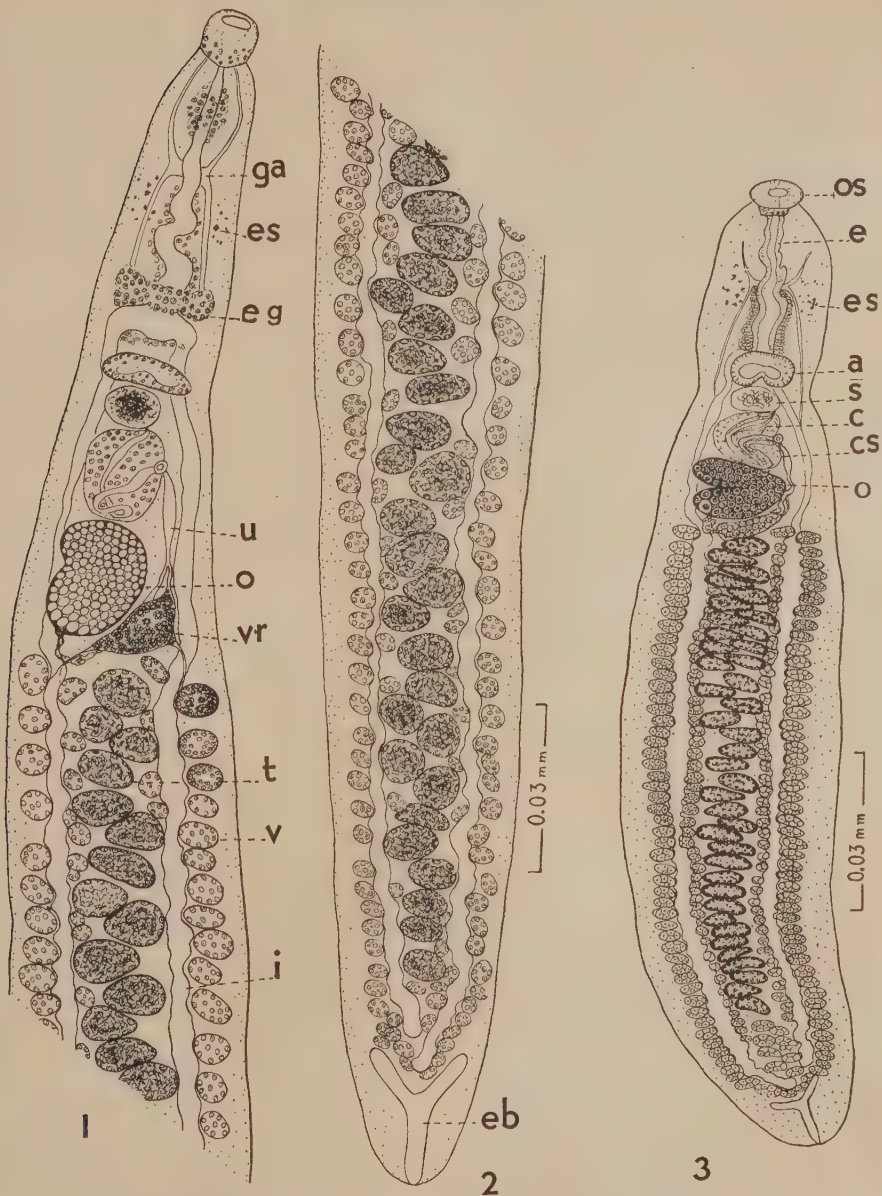
Haemoxenicon n. g.

Generic diagnosis: Spirorchidae: Small, slender trematodes. Cuticula unarmed except for small spines on margins of oral and ventral suckers. Oral sucker terminal, ventral sucker weakly muscular, retractable into body. Esophagus slender, provided with glands. Bifurcation of gut at or a short distance anterior to acetabular level. Separate ceca terminate a short distance anterior to arms of excretory bladder. Excretory bladder Y-shaped. Cirrus pouch well-developed, containing a short cirrus, tubular seminal vesicle and prostate cells; external seminal vesicle saccular, anterior to cirrus pouch. Testes postovarial, intercecal, in single or double row; numerous and variable in number. Ovary immediately posterior to cirrus pouch, weakly to conspicuously bilobed. Expanded portion of oviduct serving as a seminal receptacle. Minute pore opening on dorsal surface to left of median sagittal plane communicates with a short Laurer's canal. Uterus short. Vitelline follicles along intestinal ceca from a short distance posterior to ovary to the crura of the excretory bladder. Vitelline reservoir near posterior border of ovary. Common genital pore opens on ventral body surface to the left of median sagittal plane approximately mid-way between acetabulum and ovary. Remnants of eyespots may be present.

Type species: Haemoxenicon stunkardi n. sp.

Haemoxenicon stunkardi n. sp.

Species diagnosis: With characters of genus. Body elongate, 3.34 to 3.38 mm. long and 0.30 to 0.36 mm. in maximum width. Oral sucker terminal, 0.083 mm. in diameter. Ventral sucker approximately 0.15 mm. in diameter, pedunculated, frequently folded or even retracted into body. Acetabulum 0.55 to 0.58 mm. from anterior end of body. Margins of both suckers armed with short rows of small spines. Mouth leads to tubular esophagus which is lined with villi along most of its length. Glands present along esophagus and especially immediately anterior to bifurcation of gut (Fig. 1). Gut bifurcation a short distance anterior to acetabulum. Thin-walled ceca terminate near the crura of the excretory bladder. The left cecum always a little longer than the right. A conspicuous ganglion, located near the mid-esophagus level, gives off anterior and posterior nerves. Remnants of eyespots are present. Ovary bilobed, 0.17 to 0.21 mm. in length and 0.12 to 0.14 mm. in width. The oviduct arises from the posterior margin of the ovary, coils about, receives a small pouch-like Laurer's canal (whose pore opens dorsally), then proceeds anteriorly on the left side of the body, receiving the duct from the vitelline reservoir at the mid-ovarian level. From this point the uterus proceeds anteriorly to the common genital pore which opens ventrally at a level about mid-way between the acetabulum and the ovary. None of the specimens contained eggs. The vitellaria are spherical to subspherical and extend along the ceca from a short distance posterior to the ovary to slightly posterior to the terminations of the ceca. The vitellaria varied from 0.024 to 0.07 mm. in length and 0.012 to 0.06 mm. in width. The vitellarian products are temporarily stored in a reservoir located near the posterior margin of the ovary. The testes are oval to circular in outline and extend posteriorly and intercecally from a short distance posterior to the vitelline reservoir to a short distance anterior to the terminations of the ceca. Their size varied from 0.036 to 0.14 mm. in length and 0.024 to 0.095 mm. in width. The number of testes varied from 35 to 46. In one specimen the posterior ten testes showed signs of degeneration. They were represented by thin-walled sacs containing small masses of cells suspended in a clear fluid. The seminal vesicle is oval in outline, thin-walled, and is located immediately posterior to the acetabulum. It communicates with a slender tube containing sperm which is enclosed by the cirrus sac. The cirrus sac is well-developed, measuring from 0.17 to 0.24 mm. in length and 0.12 to 0.20 mm. in width, extending from the seminal vesicle to the ovary. It contains "prostate" cells and a cirrus. The cirrus can be extruded a short distance through the common genital pore. The excretory bladder is Y-shaped and moderately thick-walled. The stem measured from 0.08 to 0.13 mm. in length and the crura from 0.07 to 0.08 mm. in length.



FIGS. 1 and 2. Ventral view of *Haemoxenicon stunkardi.*
FIG 3. Ventral view of *Haemoxenicon chelonenecon.*

Host: Chelonia mydas.
Location: Mesenteric veins.
Locality: Pacific Ocean off Baja, California.
Type specimen: Hancock Parasitology Collection No. 499.
This species is named in honor of Professor H. W. Stunkard.

Haemoxenicon chelonenecon n. sp.

Species diagnosis: With characters of genus. Body elongate, usually exhibiting a slight constriction at or near the acetabular level, 1.38 to 1.90 mm. in length and 0.36 to 0.37 mm. in maximum width. Oral sucker terminal, 0.06 to 0.08 mm. in diameter. Ventral sucker approximately 0.12 mm. in diameter, 0.18 to 0.30 mm. posterior to anterior end of body, pedunculated, capable of being folded and retracted into body (Fig. 4). Margins of both suckers armed with short rows of small spines (Fig. 6). Esophagus tubular, lined with villi along most of its length (Fig. 5), and provided with glands. Bifurcation of gut at or a short distance anterior to acetabular level. Ceca slender, thin-walled, and extending posteriorly to near the crura of the excretory bladder. Right cecum usually slightly longer than the left. A conspicuous ganglion, located near the mid-esophagus level, gives off anterior and posterior nerves. Remnants of eyespots present. Ovary bilobed, 0.08 to 0.14 mm. along one axis and 0.11 to 0.16 mm. along the other axis. Ovary near the posterior end of the anterior one-third of body. Oviduct arises from posterior margin of ovary, slightly convoluted, receives a short pouch-like Laurer's canal (whose pore opens dorsally to the left of the median sagittal plane), then proceeds anteriorly to the left of the ovary where it receives the vitelline duct at the mid-ovarian level. From this point the uterus proceeds anteriorly to the common genital pore located ventrally to the left of the median sagittal plane approximately mid-way between ovary and seminal vesicle. One specimen, which was serially sectioned, contained an egg in the uterus. The egg, including a spine at one end, had an over-all length of 0.146 mm. and a maximum width of 0.037 mm. The spine was 0.022 mm. long. The vitellaria are oval in outline, 0.024 to 0.06 mm. transversely and 0.012 to 0.036 mm. antero-posteriorly, and extend along the ceca from near the ovarian level to a short distance posterior to the cecal terminations. A tubular vitelline reservoir lies close to the posterior margin of the ovary. Testes oval, intercecal, extending from the vitelline reservoir to near, but not reaching, the cecal terminations, and measuring 0.024 to 0.048 mm. in the antero-posterior axis and 0.10 to 0.12 mm. transversely. The number of testes varied from 37 to 44. The saccular, thin-walled seminal vesicle is located immediately posterior to the acetabulum. It communicates with a slender tube containing sperm which is enclosed by the cirrus sac. The well-developed cirrus sac, located between the seminal vesicle and the ovary, measures approximately 0.14 by 0.10 mm. It contains "prostate" cells and a cirrus. The cirrus is covered with villi (Fig. 4). The excretory bladder is Y-shaped with a terminal pore. The crura usually are about the same length as the stem (0.06 mm.).

Host: Chelonia mydas.

Location: Mesenteric veins.

Locality: Pacific Ocean off Baja, California.

Type specimen: Hancock Parasitology Collection No. 4910.

DISCUSSION

The genus *Haemoxenicon* appears to be most closely related to *Carettacola* Manter and Larson, 1950, recovered from *Caretta caretta* at the Biological Laboratory of the Carnegie Institution, Tortugas, Florida. The important differences are: a common genital pore, a minute dorsal Laurer's pore, which could be seen only in serial sections, and no vagina in *Haemoxenicon* while *Carettacola* has separate male and female pores and a large thick-walled "vagina" which opens on the surface of the right side of the body. The egg of *Haemoxenicon chelonenecon* seems to be more elongate than that of *Carettacola bipora* but since only one egg was found within *H. chelonenecon* further observations are needed. Many eggs of this type

FIG. 4. Parasagittal section of *H. chelonenecon.*

FIG. 5. Frontal section through anterior end of *H. chelonenecon* showing esophageal villi.

FIG. 6. Section showing short rows of spines characteristic of acetabular margins of both species.

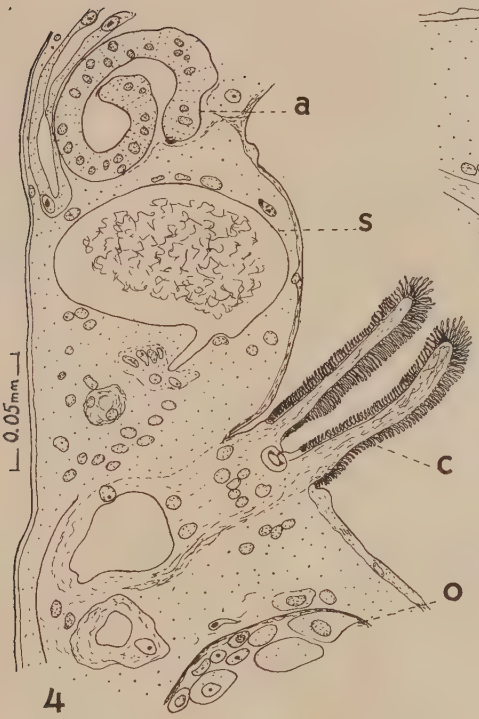
FIG. 7. Diagram of portions of the genital systems characterizing both species.

FIG. 8. Parasagittal section of *H. chelonenecon* showing common genital pore.

EXPLANATION OF PLATES

Abbreviations: a acetabulum, c cirrus, cs cirrus sac, e esophagus, eb excretory bladder, eg esophageal glands, es eyespot remnant, g genital pore, ga ganglion, i intestine, l pore of Laurer's canal, n nerve tissue, o ovary, os oral sucker, s seminal vesicle, t testis, u uterus, v vitellaria, vr vitelline reservoir.

All drawings made with the aid of a camera lucida except figure 7.



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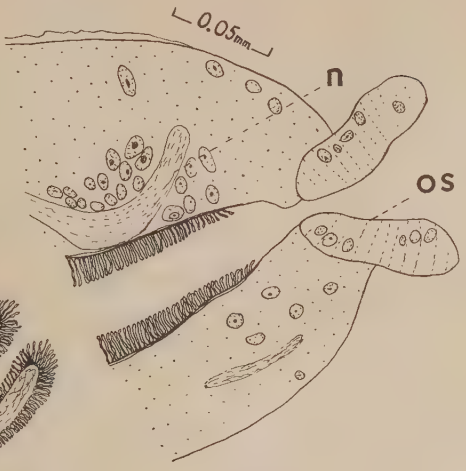

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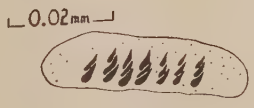

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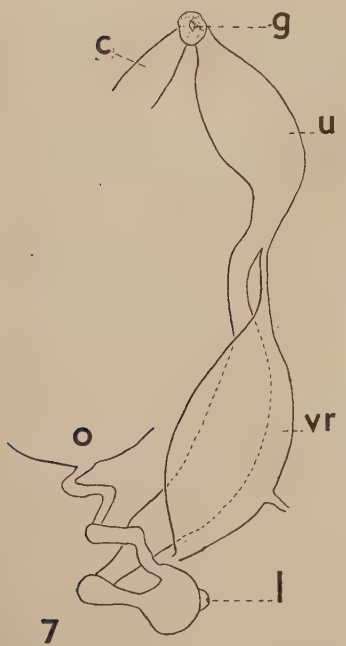

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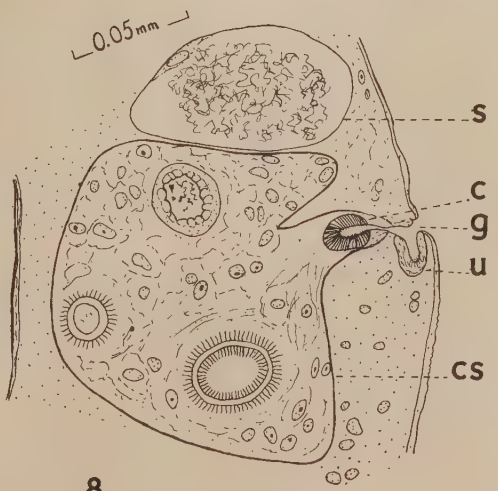

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were observed in the tissues of the lung, heart, spleen, liver, etc., but there was no way of determining if they were produced by *H. stunkardi* or *H. chelonenecon.* Also, many eggs with a spine at each end, similar to those reported by Canton (1861) attached to the conjunctivae of turtle's eyes and by Leared (1862) from a turtle's heart, were found in various tissues.

The main difference between *Haemoxenicon stunkardi* and *H. chelonenecon* is the greater length of the former. The shortest *H. stunkardi* is 1.7 times the length of the longest *H. chelonenecon.* There is no gradation in size from *H. chelonenecon* to *H. stunkardi* in living or fixed material. There is also a difference in body shape as shown in Figures 1, 2 and 3 which was evident in both living and fixed material. Therefore, the authors believe that two distinct species are involved.

SUMMARY

A new genus, *Haemoxenicon,* and two new species, *H. stunkardi* and *H. chelonenecon,* are described from the mesenteric veins of a marine turtle, *Chelonia mydas,* collected in the Pacific Ocean off the coast of Baja, California.

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THE BIOLOGY AND LIFE HISTORY OF *MONOECOCESTUS* BEDDARD, 1914 (CESTODA: ANOPLOCEPHALIDAE) FROM THE PORCUPINE[1, 2, 3]

REINO S. FREEMAN
University of Minnesota, St. Paul[4]

The Canada porcupine *Erethizon dorsatum* (Linnaeus, 1758) is rarely without the cestode parasites *Monoecocestus americanus* (Stiles, 1895) Fuhrmann, 1932 and *Monoecocestus variabilis* (Douthitt, 1915) Freeman, 1949 (see Figs. 1 and 2). Based on the morphology of the adult worm, most workers have grouped these cestodes with those from other herbivores (primarily rodents and ungulates) in the family ANOPLOCEPHALIDAE Fuhrmann, 1907 (see Freeman, 1949). Stunkard (1937 et seq.) reported that *Moniezia expansa,* from sheep, was capable of utilizing an oribatid mite as the intermediate host, and as he had earlier anticipated (1934), the life cycle proved to require only two hosts. Since Stunkard's important discovery, five other genera of anoplocephalids have been shown to develop in oribatid mites (see Kates and Runkel, 1948). However, the definitive hosts in all the life cycles investigated have been domestic grazing animals, except for the rabbit and *B. studeri* from monkey. Even the rabbit is more or less a grazer in that it depends largely on grass and clover for summer forage. Thus it becomes of scientific interest to determine whether the porcupine cestodes have life cycles like those already investigated from this family, or whether the arboreal habitat of the porcupine requires any major modifications in the established pattern for anoplocephaline life cycles.

Stunkard (1934, 1944a) thoroughly reviewed the extensive literature pertaining to the earlier studies on anoplocephaline life cycles. Kates and Runkel (1948) summarized in tabular form the known anoplocephaline life cycles as to intermediate host, author of report and geographic region of the study. Freeman (1949) discussed the taxonomy of porcupine cestodes and gave a preliminary note on the life cycle. Joyeux and Baer (1945) determined the life cycle of *Catenotaenia pusilla,* placed by most workers in the family DILEPIDIDAE. Since they found that this worm develops in various grain mites (TYROGLYPHOIDEA) they suggested that it is more closely related to species included in the ANOPLOCEPHALIDAE. Conversely, Rendtorff (1948) found that several beetles serve as intermediate hosts of *Oöchoristica ratti,* a worm usually placed in the ANOPLOCEPHALIDAE; oribatid mites failed to become infected.

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[1] Paper No. 2670 Scientific Journal Series, Minnesota Agricultural Experiment Station, St. Paul.

[2] A part of a thesis submitted to the graduate faculty of the University of Minnesota in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

[3] The author wishes to extend his thanks to all the people who aided in this study, particularly to Dr. Franklin G. Wallace under whose guidance this study was made, to Dr. William H. Marshall and others who provided the porcupines, and to Dr. Edward W. Baker of the U. S. National Museum and Dr. Joseph Bequaert of the Museum of Comparative Zoology at Harvard College for assistance in identification of the oribatid mites.

[4] Present address Southern Illinois University, Carbondale, Illinois.

The larval development of certain anoplocephaline cestodes takes place in oribatid mites. Since an imposing number of other possible intermediate hosts has failed to support such larval development (see Daubney, 1932; Krull, 1940), investigation of oribatid mites as possible intermediate hosts of *Monoecocestus* spp. was a logical place to begin.

MATERIALS AND METHODS

Collecting Mites

The most favored methods of collecting microfauna from leaf-litter utilize modifications of the Berlese funnel (for descriptions of typical funnels see Jacot, 1932, 1936; Starling, 1944; Haarløv, 1947). The following procedure was employed to obtain most of the mites for the present study. Leaf-litter, or the material from which the microarthropods were to be removed, was placed on a screen about halfway down the body of the Berlese funnel. At the upper end of the funnel was placed a 150-watt clear electric bulb in a white enamel reflector. The mites were recovered in a dry collecting bottle secured to the bottom of the funnel during a 24-hour period of operation. The tedious task of picking, sorting and identifying each mite under the dissecting microscope could not be avoided, however the mites were concentrated. A 00 camel's hair brush occasionally wetted was found effective for handling mites.

All the mites used were field-collected adults obtained from deciduous leaf-litter using the procedure described. All were collected during the summer at the University of Minnesota Biological Station, Itasca State Park, Minnesota, during 1947 through 1949, except for one series collected from St. Croix State Park, Minnesota, and some *Ceratozetes* sp. collected in St. Paul, Minnesota, during the spring of 1949. The mites used for tapeworm feeding studies were collected away from known porcupine dens and "resting trees," while mites examined for natural infections were collected near such dens or trees. Nymphs and larvae were not investigated.

The following list includes only those mites which were examined as possible vectors of *Monoecocestus*. A complete list of all mites collected during this study as well as a description of *Liacarus itascensis* n. sp., is to be published separately; this paper will include also a full bibliography on the oribatids which is omitted here. Names of mites which Dr. Edward Baker found to agree with types deposited at the U. S. National Museum in Washington or which the present author found to agree with types at the Museum of Comparative Zoology at Harvard College are followed by an asterisk; where some question remains as to the validity of the diagnosis the citation is followed by a question mark. The names used represent the latest or, in the author's opinion, the most valid combination encountered in the literature.

Achipteria armatum (Banks, 1895) Jacot, 1938*
Adoristes poppei (Oudemanns, 1906) Hull, 1916
Balzania microptera (Canestrini, 1896) Jacot, 1929 (?)
Belba Heyden, 1826
Cepheus corae Jacot, 1928*
Ceratoppia bipilis spinipes (Banks, 1906) Jacot, 1936*
Ceratozetes Berlese, 1908
Damaeus globifer Ewing, 1913
Eremaeus brevitarsus (Ewing, 1917) n. comb. (?)

Fuscozetes Sellnick, 1928
Galumna emarginata (Banks, 1895) Ewing, 1939*
Galumna nigra (Ewing, 1909) Ewing, 1938
Liacarus itascensis n. sp.
Liacarus parvulus Banks, 1909*
Neoribates quadrisetosus (Ewing, 1917) Jacot, 1929*
Scheloribates lanceoliger Berlese, 1908
Zygoribatula curviseta (Ewing, 1909) n. comb. (?)

In two cases (*Ceratozetes* and *Fuscozetes*) more than one species was present in the population. Since these species were difficult to separate under the dissecting microscope only the genus is listed. It is possible that the *Belba* listed represents an undescribed species. Several species belonging to the unusual family PHTHIRACARIDAE were tested, but since none proved capable of acting as a vector for *Monoecocestus* they were not identified to genus and species.

Laboratory Maintenance of the Mites

Most workers have stated that the methods used for the laboratory maintenance of oribatid mites left much to be desired (Krull, 1939b). Apparently Stunkard (1938, 1944b) has not published any information regarding the more recent techniques he used. During his European studies (1941) the culture methods were essentially the same as those reported by Krull (1939b); mites were maintained in culture dishes kept at room temperature and provided with abundant food and moisture. It was necessary to transfer mites to clean dishes every few days due to excessive mold growth. A simple and effective technique used in the present study was as follows. Three different sized containers with tight unperforated lids were used as growth chambers, one-pint Mason jars for stock cultures, and 30-cc. wide-mouth bottles or 15-cc. homeopathic vials for experimental studies. The number of mites was restricted to 50 or fewer per vial and 100 or fewer per bottle, while up to 400 mites were successfully maintained in a one-pint jar. The bottles and vials were lined with filter paper and a small amount of small-sized pieces of leaf-litter was added for food and cover; the pint jars had approximately an inch of litter in the bottom and no filter paper. The leaf-litter was previously prepared by crumbling and screening through a coarse screen and then heating for two hours at 180° C. for the purpose of killing other microfauna. The containers were kept at constant temperatures of 0, 5, 10, 15, 20 and 25° C. and were so maintained that some condensation of water on the wall of the container (nearly saturated humidity) could be seen at all times. Mold developed in all containers maintained at temperatures from 5 to 25° C., but only at 15 to 25° C. did it interfere with maintenance of the mite cultures. Even at these higher temperatures it could be controlled by thoroughly shaking the leaf-litter, or mixing it with an applicator stick, every week or so. To prevent asphyxiation, cultures kept at temperatures of 15° C. and higher were aerated every other day and a few drops of water were added whenever it was noted that there was no longer water condensation on the walls.

The procedures given above were successful in maintaining *Liacarus itascensis*, and were at least fair for some other species like *Scheloribates lanceoliger, Neoribates quadrisetosus* and *Belba* sp. As confirmation of Krull (1939b), *Galumna* spp. were found very difficult to maintain, although a few were kept long enough to demonstrate that they are capable of serving as intermediate hosts of *Monoecocestus*.

All evidence indicates that no one set of laboratory conditions will be found satisfactory for maintaining a group of mixed species such as might be gathered from a batch of leaf-litter.

Mites were not maintained satisfactorily in the laboratory at 25° C., but mites have been kept at 15 and 20° C. for over three months, for four and one-half months at 10° C. and over nine months at 5° C. In fact it was found that 5° C. with a nearly saturated humidity was a good holding condition for all species of the ORIBATEI. No mite eggs or larvae were observed in colonies kept at temperatures under 15° C., while some were observed in cultures between 15 and 20° C.

Two preliminary trials at rearing mites in the laboratory were unsuccessful. They were not pursued further when it became clear that the use of field-collected adults in the tapeworm experiments could be justified.

Collecting and Feeding the Cestode Eggs

All the cestode eggs used in the feeding studies were obtained from worms removed from naturally infected porcupines. A porcupine was sacrificed every time cestode eggs were desired because no way was found to purge the animals of their tapeworms. No attempt was made to use eggs directly from the feces since ample numbers of infected animals were always available and because eggs from identified cestodes were desired.

Eggs from the posterior 5 to 10 proglottids of tentatively identified worms were removed and the remainder of the worm was fixed for later positive identification. A sample of these eggs was examined microscopically for viability. If viable the remainder was placed onto a filter paper. The paper was dried until only slightly moist to the touch and stored in a stoppered vial in a refrigerator for a maximum of 48 hours.

Mites were exposed to tapeworm eggs as follows. The paper with the tapeworm eggs was air dried until it felt dry to the touch, and was then placed in a feeding vial. Approximately 100 identified mites were placed in the vial, the vial corked and set aside at room temperature. The vial was examined several hours later to check the moisture content. It was observed that for maximum feeding activity the vial should have only a trace of water condensation on the wall. The feeding activity was followed several times under the dissecting microscope.

Mites exposed to tapeworm eggs longer than 48 hours did not acquire appreciably heavier rates of infection than did mites exposed for 48 hours; mites exposed for 24 hours had noticeably lighter rates of infection.

Procedures in the Study of the Cestode Larvae

The mites were dissected in a drop of saline under a dissecting microscope. A stock saline (modified from Yeager, 1939) with 2.7 gm. of sodium chloride, 0.4 gm. of potassium chloride, 0.2 gm. of calcium chloride and 0.05 gm. of sodium bicarbonate in a liter of distilled water was used. Before covering a preparation with a cover glass, a drop of distilled water was added. A "cold" fluorescent lamp as a dissecting light was used to minimize the rate of evaporation. All measurements of cestode larvae were taken as soon as possible after the dissection. Distilled water had to be added every 10 or 15 minutes if the larvae were to be kept from desiccation. The changing tonicity was not always harmful, for many morphological features (e.g., flame cells) were best revealed after changes in tonicity. Some fully developed

larvae were kept alive under a cover glass ringed with vaseline up to ten hours, and after apparent death they sometimes did not begin to show gross morphological deterioration for another 30 hours. Intra-vitam staining with neutral red and Nile blue sulfate, ready prepared in the dissection saline, was used to supplement the unstained living material. Specimens fixed in Bouin's or FAA and later stained in carmalum or hemalum were studied, but were not as satisfactory as the living material.

RESULTS

Mites Infected

Field-collected mites were used exclusively in these studies, but their use was justified by adequate controls. Controls were not kept during 1947 when the tapeworm feeding studies were first begun, but in 1948 and 1949 controls from the same collections as the experimental mites were maintained. Nearly one thousand control mites were examined in all, and only one (a single *Neoribates quadrisetosus* out of 54 examined in 1948) was found infected (Table 1). From this same batch of mites 31 out of 69 individuals exposed in the laboratory were found infected, so

TABLE 1.—*A summary of dissections of naturally and experimentally exposed mites*

Species	Naturally exposed mites		Experimentally exposed mites: Monoecocestus 1947		M. americanus 1948		M. americanus 1949		M. variabilis 1948		M. variabilis 1949		Controls 1948	Controls 1949
	D*	I*	D	I	D	I	D	I	D	I	D	I		
Achipteria armatum	13	0	2	0	1	0	1	0	4	0	0	0	35	
Adoristes poppei	17	1	1	0	11	1	0	0	3	0	0	0	22	
Balzania microptera	3	2	0	0	0	0	3	0	0	0	0	0	4	
Belba sp.	10	0	6	0	7	2	14	4	1	0	18	0	29	
Cepheus corae	43	0	22	0	1	0	3	0	24	0	2	1	16	
Ceratoppia bipilis spinipes	39	0	7	1	18	8	0	0	11	6	9	1	15	
Ceratozetes sp.	10	0	0	0	0	0	37	0	1	0	0	0	3	
Ceratozetes (St. Paul)	0	0	0	0	0	0	36	0	0	0	0	0	0	
Damaeus globifer	1	0	13	0	0	0	5	1	0	0	0	0	23	
Eremaeus brevitarsus	1	1	24	0	1	1	6	5	0	0	0	0	7	
Fuscozetes spp.	14	1	13	0	12	2	9	0	0	0	0	0	84	
Galumna emarginata	128	0	104	2	1	0	14	4	18	0	0	0	22	
Galumna nigra	2	0	3	2	64	15	0	0	58	3	5	1	93	
Liacarus itascensis	119	4	11	1	32	9	312	88	31	1	236	69	167	119
Liacarus parvulus	20	0	1	0	7	0	0	0	0	0	0	0	21	
Neoribates quadrisetosus	35	0	14	8	35	24	13	3	34	7	4	0	53 + 1**	42
Phthiracaridae	23	0	2	0	27	0	0	0	10	0	0	0	42	
Scheloribates lanceoliger	21	1	21	0	8	2	223	14	3	0	0	0	92	
Zygoribatula curviseta	16	2	0	0	0	0	0	0	7	0	4	0	0	
Unknowns and miscellaneous	5	0	25	0	1	0	6	0	1	0	2	0	39	

* D—number of mites dissected; I—number of dissected mites found infected.
**—the only control found infected.

the probability of error introduced by natural infections was regarded as insignificant.

Table 1 is a summary of all dissections of live mites, tabulated by years. Fully formed cysticercoids were found in at least one specimen of each species reported as positive, except for *Cepheus corae,* where only a partially developed cysticercoid was observed. In all, 14 out of 19 species of mites adequately tested have been found infected with *Monoecocestus* spp. Eight species (including one control) were found naturally infected; eleven species were successfully experimentally infected with *M. americanus* and five with *M. variabilis.* Two species of mites, *Balzania microptera* and *Zygoribatula curviseta,* found naturally infected were not successfully experimentally infected with either species of worm; both species of mites were poorly represented in leaf-litter and difficult to keep alive in the laboratory. Apparently the degree of specificity as to the intermediate host is very low.

Most of the study of larval development as well as the effects of temperature on larval development was done on larvae from *Liacarus itascensis*. Six hundred twenty-two *L. itascensis* were dissected at various times following exposure to cestode eggs, and 168 (27 per cent) were found to be infected. Two hundred eighty-six controls were negative. This percentage of experimentally produced infection is a conservative estimate of the true rate, for many mites were examined very soon after exposure to tapeworm eggs and found to be negative. In later dissections from the same series of mites, but when the tapeworm larvae were larger and easier to find, the rate of infection was always higher. Many mites were infected with more than one cysticercoid, so that the rate of infection shown above does not tell how many cysticercoids were present.

Development of Monoecocestus *spp. in the Intermediate Host*

In the following descriptions the word "stage" is used for arbitrarily defined steps in development of the larva. By comparison of these stages it is possible to ascertain the degrees of development attained by larvae after the mite hosts have been maintained under different environmental conditions for specific lengths of time. The word "stages" is not intended to connote any major change in development, comparable to onchosphere, larva and adult, as used by Stunkard (1934).

The mature egg (Figs. 3 and 4) has been described previously (Freeman, 1949); however, since some errors of measurement were inadvertently included in this description, and since additional observations have been made, a complete redescription is included here. The egg averages 60 microns in outside diameter. It is composed of the onchosphere, or hexacanth embryo, surrounded by three shells or coats. The outer coat is covered with minute spines about 3 microns in length (Fig. 3), a character apparently unique to this family of cestodes (Hadwen, 1922). The middle shell, approximately 50 microns in diameter, is subspherical and generally in contact with a portion of the outer coat. Between the middle and outer coats are numerous spherical to irregularly shaped bodies (seen extruded to the right of the pyriform apparatus in Fig. 4). These structures are oily or sometimes granular in appearance and make study of the intact egg difficult. The outer and middle shells may be ruptured by applying gentle pressure to the cover glass; the pyriform apparatus, or inner shell, containing the hexacanth embryo is extruded (Fig. 4). This inner shell averages 40 microns in length by 25 microns across the globose portion. On its narrower end is an indentation (a dark trace in Fig. 4) which separates two short blunt horns. Further growth and twisting of the horns as has been reported for some other anoplocephaline eggs (Rees, 1933; Sinitsin, 1931) were not observed. From the ends of these horns appear to issue what were previously described as "two filaments." The identity of the "filaments" remains uncertain, but they could be interpreted as the wrinkled edges or folds of an otherwise loose, transparent sheath around the narrower end of the pyriform apparatus. When a pyriform apparatus is properly oriented, a wing-like expansion may be observed projecting from the margin of the narrowed end of the pyriform apparatus (Fig. 4).

The subspherical hexacanth embryo averages 17 microns in diameter. It is surrounded by a loose membrane which occasionally can be seen while the embryo is in the pyriform apparatus (probably the same membrane standing well away from the larva in Fig. 5). The six embryonic hooks, each averaging 10 microns in length,

EXPLANATION OF FIGURES

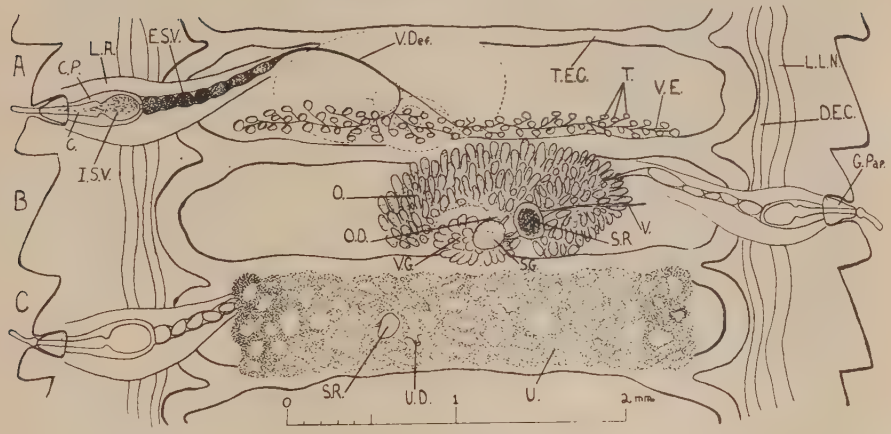


FIG. 1

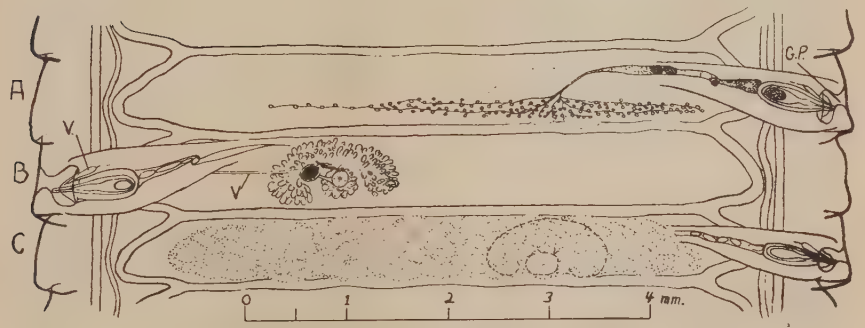


FIG. 2

PLATE I

FIG. 1. *Monoecocestus americanus* Proglottid A, semidiagrammatic representation of male reproductive structures in a mature proglottid, viewed from above; proglottid B, semidiagrammatic representation of female reproductive structures in a mature proglottid, viewed from above; proglottid C, semidiagrammatic representation of developing uterus, viewed from above. (Although the condition depicted in proglottid C usually occurs 5 to 10 proglottids more posteriorly in the strobila, for convenience it is shown here as the succeeding proglottid immediately following B.)

FIG. 2. *Monoecocestus variabilis* Proglottids A, B and C following the same pattern used in Fig. 1. C.—cirrus; C.P.—cirrus pouch; D.E.C.—dorsal excretory canal; E.S.V.—external seminal vesicle; G.P.—genital pore; G.Pap.—genital papilla; I.S.V.—inner seminal vesicle; L.A.—lax area; L.L.N.—lateral longitudinal nerve; O.—ovary; O.D.—oviduct; S.G.—shell gland; S.R.—seminal receptacle; T.—testes; T.E.C.—transverse excretory canal; U.—uterus; U.D.—uterine duct; V.—vagina; V.Def.—vas deferens; V.E.—vas efferens; V.G.—vitelline gland.

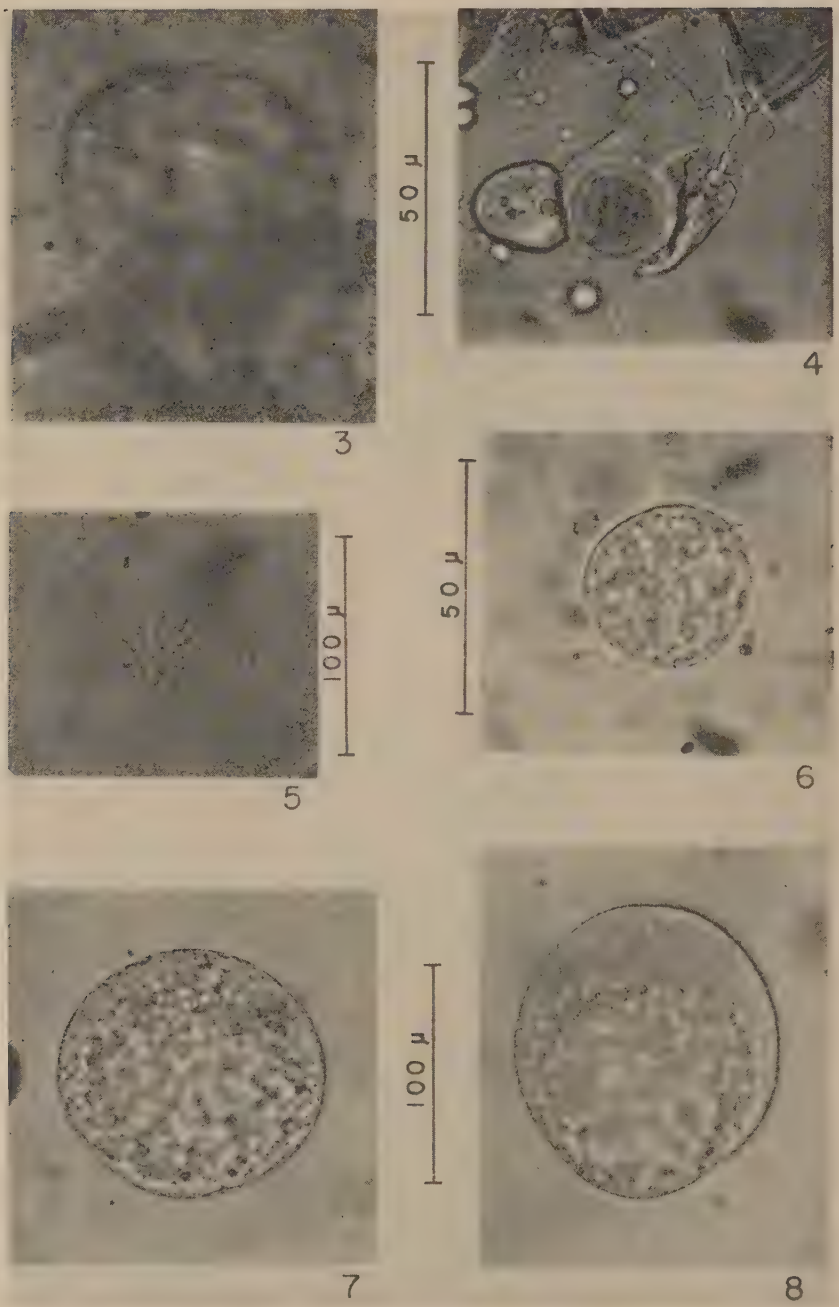


PLATE II

FIG. 3. Complete egg of *Monoecocestus* sp.
FIG. 4. Pyriform apparatus with intact onchosphere from crushed egg of *Monoecocestus* sp.
FIG. 5. Embryo, stage 1, of *M. americanus*, from haemocoel of *Liacarus itascensis*.
FIG. 6. Early morula, stage 2, of *M. variabilis*.
FIG. 7. Well-developed blastula, stage 3, of *M. variabilis*.
FIG. 8. Advanced blastula stage of *M. variabilis*.

(see Figs. 4, 5 and 6), are the most conspicuous feature of the embryo. Cellular details were difficult to observe due to the small size of the embryo, but Nile blue sulfate demonstrated some dark blue-staining bodies lateral to the bases of the outer two pairs of hooks (seen as dark areas in Fig. 4) which were interpreted as similar to the penetration glands of *Moniezia* (see Reid, 1948). Muscle fibers, flame cells and other embryonic details which have been reported from other species were not observed.

All parts of *Monoecocestus* eggs were found in the alimentary canal of mites 15 hours after their initial exposure. Krull (1939b) observed that mites did not eat the outer shells when fed eggs of other anoplocephalines. Perhaps the fact that eggs of *Monoecocestus* spp. possess spines on the outer shell facilitates their recognition in the gut of the mite. Pyriform apparatuses were observed both without embryos and with inert embryos still contained. Some embryonic hooks were observed also. No evidence was found as to where along the gut the embryo penetrated into the haemocoel, although the search for these early stages included a careful examination of the wall of the alimentary canal. The two earliest larvae were found at five days post-feeding. (Potemkina, 1944b, found *Thysaniezia ovilla* two days post-feeding.) These embryos were elongate-oval and measured 18 by 26 microns. The hooks still moved in active coordinated motion. At least two large cells, measuring 8 microns in diameter and each containing an eccentric nucleus, were observed posterior to the hooks. It is presumed that these were the germinal cells. The remainder of the embryo was a homogeneous ground substance filled with granules. Evidences of muscle fibers, flame cells and other types of cells were not observed. Such embryos have an affinity for tissue. One larva was found imbedded in ovarian tissue, and another in some tissue debris which it penetrated while under observation.

The germinal cells continue to divide until a solid sphere is formed. This is the morula, solid ball or stage two (Fig. 6). As the number of cells increases the organization of the embryo disappears; hooks first show uncoordinated movement, then cease movement altogether. The germinal cells, up to 14 microns in diameter, contain a nucleus with an eccentric nucleolus; the cytoplasm is granular. Between the cells are small greenish irregular granules. Occasionally the morula shows faint contraction. The limiting membrane is more pronounced, and the larva may be 40 to 50 microns in diameter.

The cells in the center of the morula begin to separate in the beginning of stage three, the blastula or hollow ball stage (Figs. 7 and 8). Although the cells continue to divide and increase in number, there is little decrease in the size of the cells. Coordinated with the increase in cell number is the increase in volume of the cavity in the center of the cell mass; the cavity becomes more spherical, but there is no limiting membrane lining this cavity (Figs. 7 and 8). The membrane surrounding the embryo becomes thicker, and immediately under it are traces of fibers and protoplasmic connections between the cells. The blastula is very fragile but capable of feeble contractions. The six embryonic hooks may be variously placed in the surface of the blastula, generally in pairs. The blastula may attain a diameter of 150 microns.

The cavity within the blastula becomes more eccentric in position as the larva grows older (Fig. 8). The thicker side is to produce the future scolex while from

the thinner side develops the cercomere. A bud-like swelling may push out of the thinner side (Fig. 8), or the whole blastula may elongate. The antero-posterior axis of the body is now established. Rapid cell division and general larval elongation ensue; this is stage four, the elongate or vermiform stage (Figs. 9 and 10). The peripheral cells become more compact and organized while the more centrally placed cells continue in a less organized relationship (Fig. 9). Circular muscle fibers are well developed; the body may constrict at various places, or waves of contraction may progress up or down the body. The shape of this stage is highly variable; cavities appear dependent on the position and intensity of the constrictions, although generally a cavity near the posterior end remains. Older larvae in this stage are highly vermiform, attaining over 300 microns in length.

A definite cercomere is pinched off; this initiates stage five, the segmented stage (Fig. 11). The cercomere undergoes very little further change, except to elongate or modify in shape due to contraction of subcuticular muscle fibers. The hooks of the embryo can be found in the wall of the cercomere, although occasionally they may be on the forebody. The major future development occurs in the forebody, which constricts into two parts shortly after the cercomere is pinched off (Fig. 11). In the anterior half of the forebody calcareous granules become visible near the constriction (Fig. 11). The part next to the cercomere is to become the future cyst and receive the scolex when it is retracted and invaginated. The cyst wall becomes more organized. Under a thickened cuticle muscle fibers become more pronounced while immediately beneath them, the cells which were formerly loose and generalized become compact and form a solid tissue. The cells toward the center of the cyst modify into a loose parenchymatous tissue. The entire larva is now more compact and may be reduced in size from that attained in the vermiform stage.

Noticeable changes have occurred in the scolex primordium while the cyst was developing. The number of calcareous granules has increased, and anteriorly four discrete areas, the primordial suckers, are delimited. These primordial cell aggregates are very unstable, and disappear if the tonicity of the saline is not correct. This is the beginning of the preinvagination stage, stage six (Figs. 12 and 13).

Flame cells and traces of tubules of the excretory system are visible by the time the sucker primordia are noticeable. The excretory system is fully developed when the scolex is ready to invaginate into the cyst. This system consists of four longitudinal tubules, and at the upper or anterior end of the scolex four transverse tubules unite the ends of the longitudinal tubules into a square in the area surrounded by the suckers. Branch tubules, terminated by flame cells, unite with the main tubules at irregular intervals. The longitudinal tubules traverse the length of the scolex and unite, at the juncture of the scolex and the cyst, into a short common tubule which divides into at least two tubules after it enters the cyst proper (Fig. 12). Flame cells are found in the wall of the cyst, but no tubules uniting these flame cells with the central excretory tubules were observed. The two tubules traverse the cyst to the juncture of the cyst and cercomere, where, in some cases, the tubules again unite. This single tubule enters the cercomere and appears to traverse it to the very tip. At least two flame cells are part of this single tubule. In some cases two tubules of undetermined extent appear to enter the cercomere.

Just how the suckers develop was not determined. The basic sucker morphology is established by the time the sucker cell-aggregate is so well differentiated that it

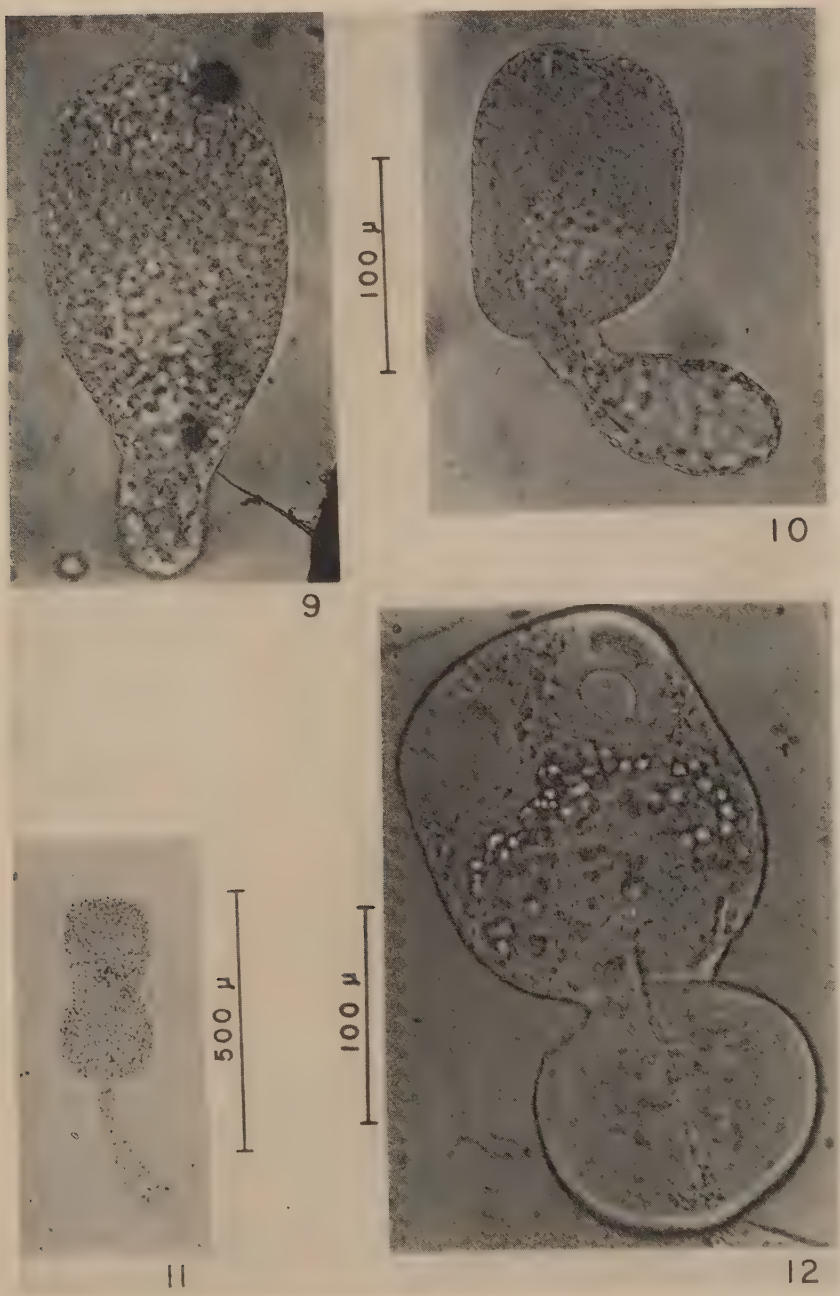


PLATE III

FIG. 9. Elongate stage, stage 4, of *M. variabilis*.
FIG. 10. Later elongate stage of *M. variabilis*.
FIG. 11. Early segmented stage, stage 5, of *M. americanus*.
FIG. 12. Well-developed preinvagination stage, stage 6, of *M. americanus*.

does not disappear with the manipulation required to get the larva ready for study (Figs. 12 and 13).

The cyst is well developed when the scolex is ready to invaginate. The wall of the cyst has three discrete layers, the cuticle or outer membrane, the circular muscle layer and the compact tissue layer, while the central region is a loose matrix of large cells and strands or fibers. These fibers, probably muscular, extend into the base of the scolex and apparently aid in the retraction process.

Stage seven, the invagination stage, includes those larvae in which an invaginal canal is seen. This canal is the opening uniting the cavity in which the scolex resides with the outside (seen at the top of Fig. 14). It results from the incomplete union of the edges of the cyst wall over the scolex. Invagination of the scolex is a complex process. The cyst fibers mentioned above contract and the scolex invaginates into its basal portion; meanwhile the invaginated scolex is retracted into the cyst. It is thus that the calcareous granules seen extending transversely across the base of the uninvaginated scolex (Fig. 12) come to surround or line the cavity in which the scolex resides after invagination (Figs. 14, 15 and 16).

The invagination canal, readily seen in newly invaginated cysts, becomes more difficult to identify in the mature cysticercoids; the mature cysticercoids constitute stage eight (Figs. 15 and 16). The forebody of the cysticercoid varies from 122 to 185 microns in diameter; after development at room temperature a diameter of approximately 160 microns is most common. The dimensions of the cercomere vary from 585 microns long by 41 microns wide to 64 microns long and 28 microns wide, although after growth at room temperature 150 to 300 microns long by 30 to 50 microns wide is most common. Uniform measurements of the suckers and scolex were difficult to obtain because of their motility. The diameter of the scolex varies from 70 to 122 microns, and that of the sucker from 30 to 60 microns. It is interesting to note that the mature and maturing worms recovered from the intestine of the porcupine had a scolex diameter of around 500 to 600 microns and a sucker diameter of around 200 microns. Mature cysticercoids were produced in as short a time as 45 days in mites kept at 25° C., but developmental times of 50 to 80 days were more common at room temperatures. It is estimated that complete development of the cysticercoid may require from 80 to 100 days in nature. A paper discussing the effect of temperature on the rate of growth and total size of *Monoecocestus* larvae is to be presented separately.

Further changes in the development of *Monoecocestus* cysticercoids have not been observed to occur in larvae kept at 20° C. for one month or at 5° C. for six months after complete development. Stunkard (1941) reported that as cysticercoids grew older the cercomere shriveled. In fact, elsewhere (1940a) he used the presence of a large cercomere as the criterion for declaring such a cysticercoid as immature. Contrary to Stunkard's findings with *Cittotaenia,* it was found that cysticercoids of *Monoecocestus* with fully developed cercomeres were infective to porcupines. It was observed also that the cercomere was very adhesive; a cysticercoid complete with cercomere was difficult to obtain unless great care was used in dissection.

In *Monoecocestus* the scolex is nearly, if not fully, developed when invagination occurs (Figs. 11 and 12), while in *Cittotaenia* the scolex becomes invaginated while the suckers are only rudiments (Stunkard, 1941). Otherwise the development is similar to that described for other anoplocephaline cestodes for which the larval

development is known (Stunkard, 1938, 1940a, 1941; Bashkirova, 1941a and b; Potemkina, 1944a and b).

The hooks of the embryos of both species of *Monoecocestus* are identical in size and shape. Their length is uniform near 10 microns; the extremes of 8 and 12 microns (Freeman, 1949) were observed only once. In Table 2 are listed the common anoplocephaline species likely to be encountered at Itasca State Park, Minnesota, with potential definitive host, length of embryonic hook and authority for the citation.

From this table it is clear that size of hooks alone is not sufficient to differentiate a *Cittotaenia* larva from one of *Monoecocestus*. The likelihood of encountering a *Cittotaenia* larva was reduced by restricting the collection of mites to be examined for natural infections to the immediate vicinity of large piles of porcupine fecal pellets. Five hundred twenty mites collected near such fecal pellets included 12 naturally infected mites, whereas 929 control mites from the general forest floor included only 1 infected mite (Table 1). This supports the contention that the natural infections reported in this study are *Monoecocestus* spp., since rabbits (and *Cittotaenia* larvae) are as apt to occur on the forest floor as near porcupine pellets.

TABLE 2.—*Length of embryonic hooks of some Anoplocephalidae*

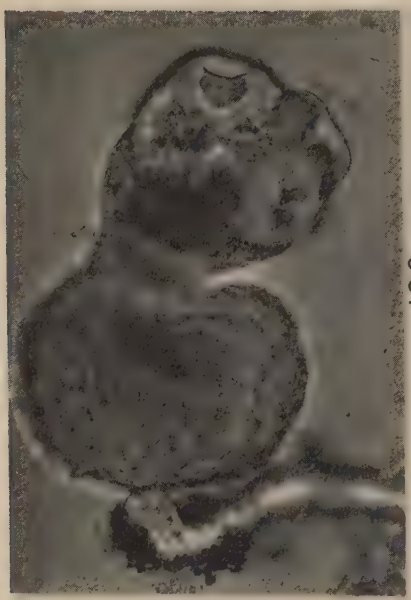
Species	Host	Length of Hook in Microns	Authority
Andrya macrocephala	Mice	5	This study
Paranoplocephala infrequens	Mice	5	This study
Moniezia benedeni	Deer	6–7	Obitz (1934)
Moniezia expansa	Deer	6–7	Sinitsin (1931)
Cittotaenia ctenoides	Rabbits	7	Obitz (1934)
Moniezia expansa	Deer	8	This study
Moniezia expansa	Deer	9	Kates and McIntosh (1950)
Moniezia benedeni	Deer	9	Kates and McIntosh (1950)
Cittotaenia spp.	Rabbits	ca. 10	Stunkard (1934)
Monoecocestus spp.	Porcupines	10	This study
Cittotaenia ctenoides	Rabbits	10–11	Stunkard (1941)

Infection of the Definitive Host

There are only two reports of *Monoecocestus americanus* or *M. variabilis* being found in animals other than the porcupine. Stiles (1895) described a subspecies of *M. americanus* from a "rabbit," and Olsen (1939) reported both species of *Monoecocestus* from the *stomach* of the muskrat. Cysticercoids, or intact mites from cultures known to contain cysticercoids, were experimentally fed to four rabbits, two hamsters, two chicks and one white rat. The cysticercoids were pipetted directly into the rear of the mouth, while the mites were either placed directly on the tongue or mixed into a pellet of alfalfa feed. None of the animals was found to be infected on post-mortem examination 11 to 63 days after feeding.

Two mature porcupines collected the summer of 1949 were available for studies that fall, but both had heavy naturally acquired infections of *Monoecocestus*. Efforts were made to eliminate or reduce the infection by anthelmintic treatment. Drugs were administered by a stomach tube while the animal was under light ether anesthesia. Oleoresin, oleoresin followed by magnesium sulfate, basic lead arsenate, and Nemural, a commercial dog taeniacide, were tested at various dosages without success.

The possibility of superimposing a new infection on the existing one in each of these two porcupines was investigated. Intact mites with known rates of infection



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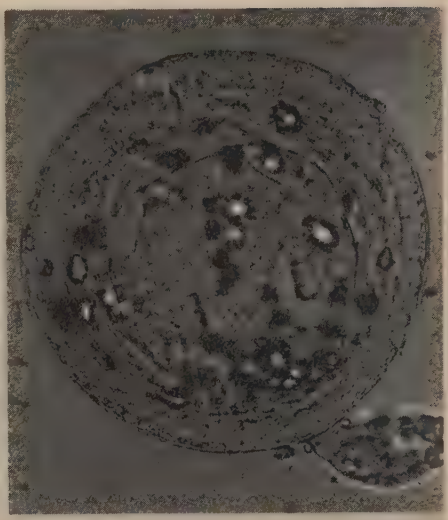

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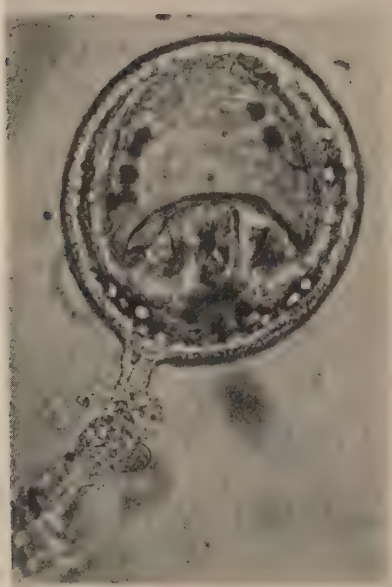

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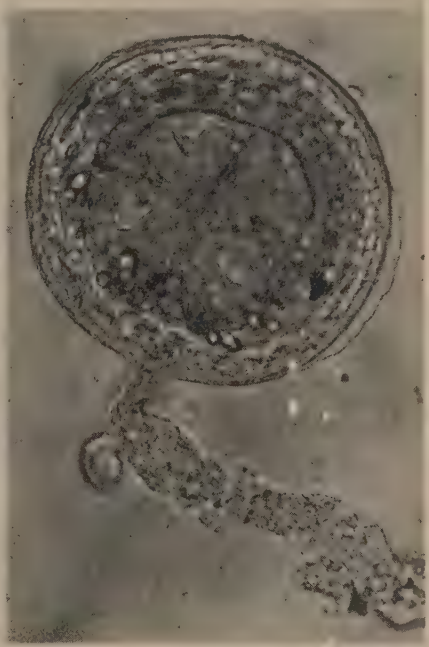

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PLATE IV

FIG. 13. Well-developed, but contracted, preinvagination stage of *M. americanus.*

FIG. 14. Late invagination stage, stage 7, of *M. americanus.*

FIG. 15. Fully developed cysticercoid, stage 8, of *M. variabilis;* scolex highly contracted and cercomere damaged.

FIG. 16. Fully developed cysticercoid of *M. americanus;* cercomere slightly damaged.

were fed by stomach tube. The results were not conclusive, for on post-mortem examination approximately three weeks after exposure two clear-cut age groups of worms were not recovered.

To exclude the possibility of accidental infections through insect hosts certain studies were made. Both of the experimental porcupines were heavily infested with the porcupine biting-louse, *Eutricophilus setosus* (Giebel, 1861) (see Jellison, 1933). Over 400 lice from these porcupines were dissected but no cestode larvae were found. Grain beetles could be excluded, because the way the food was handled precluded the possibility of those grain beetles which might have been exposed to tapeworm eggs being later available to the porcupines. Krull (1940) had negative results with cockroaches exposed to *Moniezia;* experiments to test cockroaches with *Monoecocestus* eggs were not carried out. The way the experimental porcupines were maintained in the laboratory excluded the possibility of their eating other arthropods which might have had access to the cestode eggs in the feces of porcupines.

In May of 1949 three female porcupines which had been captured earlier that spring each gave birth to one young. The young were allowed to remain with their respective mothers for about two months. The only solid food fed to the young or the mothers was a commercial rabbit food consisting of pellets of alfalfa concentrate. These three laboratory-reared porcupines, designated Uno, Duo and Tre, were used for critical feeding experiments carried out the following fall.

Duo was fed, by stomach tube, 50 *Liacarus itascensis* in which *M. variabilis* was known to occur at the rate of 1.5 cysticercoids per mite. Uno was designated as a control. Duo was negative when examined 52 days after the experimental feeding. The control was not sacrificed since the principal was negative.

This negative animal demonstrated that infection did not occur by the direct consumption of cestode eggs since all three young were exposed to cestode eggs while with their mothers. It appeared, therefore, that either the technique of administration was faulty or that the larvae in question were not infective to porcupines. It was possible that the cysticercoids were unable to get out of the mite since by the method used the mite was introduced directly into the stomach undamaged. To test this hypothesis the procedure was modified as follows. Uno, previously the control, became the principal of the second experiment. It was fed 45 *L. itascensis* from the same stock culture as had been fed to Duo previously. The mites were crushed between a cover glass and slide; each mite was examined under the dissecting microscope to be sure the cuticle was cracked. The mites were fed by stomach tube as previously. Uno was examined 44 days after the feeding and at least 21 short tapeworms were recovered; of these, seven retained the terminal proglottid, while the remainder had one or more of the posterior proglottids missing. The worms were all juvenile and the more complete worms measured from 15 to 31 mm. in length by 2.5 to 4.5 mm. in width. The worms were too immature to ascertain the species with any degree of certainty.

Tre was fed 50 crushed *L. itascensis* which had been exposed to *M. americanus* eggs; the culture from which the mites were taken had previously been determined to have an infection rate of 1.1 cysticercoids per mite. When sacrificed 70 days later Tre contained 25 fully mature worms, all identified as *Monoecocestus americanus.* These worms ranged in length from 72 to 193 mm. and in width from 5 to 10 mm.; the average adult was 124.5 by 7.2 mm. The worms were sexually mature and

viable ova were found in the ripe proglottids of those examined. The rate of infection reported here is higher than that obtained by previous workers with other anoplocephalines (Krull, 1939a; Shorb, 1939; Stunkard, 1941). It is possible that other failures to produce infection when feeding intact mites would have resulted otherwise if the mite host had had the exoskeleton damaged.

The possibility of superimposing a new infection on an old one was reexamined when it was discovered that the original technique was faulty. The results obtained were no more conclusive than those reported above.

No evidence for a tissue-invasive stage in the definitive host during the early phases of the infection, postulated by Stoll (1937) and Evans (1940) and vigorously refuted by Stunkard (1938, 1941), was found in the present study.

Infection of the Porcupine in Nature

In winter the porcupine is arboreal (Taylor, 1935); however, in other seasons it spends much of its time on the ground. Generally it remains close to its feeding area. It is common for several porcupines to frequent the same den or resting place at different times, even though they are primarily solitary in habit (Gabrielson, 1928). In some parts of the country, dens are located by the more luxuriant growth of vegetation near the entrance (Taylor, 1935).

Taylor (1935) made extensive studies on food habits of the porcupine. He found from analyses of 70 adult and 14 young porcupine stomachs that herbaceous ground vegetation is the primary summer food. He concluded that the porcupine is more of a browser than a grass eater, although grass was found in the stomachs on occasion. The only porcupine observed feeding in nature during the present study was eating raspberry leaves. Examination of the ground vegetation immediately surrounding one of three dens at Itasca State Park showed many of the plants to have leaves neatly cut off, apparently due to porcupine cropping.

The porcupine is a very thorough masticator once a particular piece of vegetation is selected. The present observations corroborate those of Taylor when he says: "The porcupine masticates with an audible chopping sound, reminding one of a little pig. The material is often finely chewed, so as to make identification in the stomach quite difficult."

It would appear that conditions in nature are ideal for the successful completion of a *Monoecocestus* life cycle. Porcupine feces containing proglottids of *Monoecocestus* are concentrated into a limited area. The feces stimulate heavy plant growth, which creates leaf-litter conducive to mite populations. The mites migrate up and down the ground vegetation. Generally the heaviest concentrations of mites on the vegetation occur at night and during the morning twilight, the very times when porcupines are eating. Porcupines are not loath to eat the vegetation immediately adjacent to their dens, where infected mites are most likely to occur. The careful mastication of the vegetation by the porcupine increases the likelihood that the mite's box-like exoskeleton will be damaged before the mite is swallowed.

Transfer of infection from host to host is assured by the porcupine habit of frequenting communal dens. The chance of a young animal being infected is further enhanced because the first few months of its life are spent on the ground, eating solid foods a week to ten days following birth (Taylor, 1935).

That *Monoecocestus* spp. have a life history remarkably adapted to that of the

porcupine is further demonstrated by the high percentage of the latter found infected in nature. During the present study 23 of the 24 animals examined contained tapeworms. One animal, badly injured on capture, had no cestodes when examined, although it passed proglottids when first captured. Of 74 porcupines recorded as examined by previous workers at the Parasitology Laboratory of the Division of Entomology and Economic Zoology, University of Minnesota, 65 were infected with cestodes. Two of the 9 without cestodes were obviously sick before autopsy; no accurate information was available to indicate the age or general health of the other 7 animals. The present study lends support to Taylor's (1935) observations that often infected animals appear healthy, while uninfected animals appear sickly. Taylor found young animals frequently uninfected. During the present study two young animals caught in the forest and estimated on weight and size basis to be three to four months old, had 3 and 25 cestodes respectively. Wild mammals rarely show a rate of infection by any helminth as high as here shown for these cestodes.

SUMMARY

1. Maintenance of oribatid mites under artificial conditions is described.

2. In all, 14 species of oribatid mites representing 12 genera were found infected with *Monoecocestus* spp. *M. americanus* was reared from the egg stage to the cysticercoid stage in 11 species of mites representing 10 genera, and *M. variabilis* was similarly reared in 4 species of mites representing 4 genera. One species of mite representing a separate genus was found with an incompletely developed larva of *M. variabilis*. Naturally acquired cysticercoids were found in 8 species of mites from 8 genera; 2 of these 8 species found naturally infected failed to become infected after laboratory exposure.

3. The development of *Monoecocestus* spp. in the oribatid mites is described.

4. Recognition of *Monoecocestus* sp. larvae in natural infections is discussed.

5. Laboratory-raised porcupines were infected with experimentally reared cysticercoids of both species of cestodes; attempts to infect rabbits, hamsters, chicks and a white rat were unsuccessful.

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PARASITISM OF MOSQUITO LARVAE BY MERMITHIDS (NEMATODA)

ROBERT M. STABLER
Colorado College, Colorado Springs

Numerous species of fungi, bacteria, and worms have been recorded as parasitizing mosquitoes. Some have been shown to be definitely destructive of these insects (for a bibliography of the relevant literature up to 1927 see Speer, 1927). Among the worms, there are nematode parasites of adult mosquitoes, such as those described from *Aedes sollicitans* by Stiles (1903), by Smith (1904), and those from *A. vexans* females by Hearle (1926). These latter, collected first in 1920 and 1921 by Hearle, were called *Paramermis canadensis* by Steiner (1924), and were said to interfere with ovarian development. Stiles (1903), who was the first to describe a mermithid worm from a mosquito in North America, proposed the name *Agamomermis culicis* for his form. Other similar worms are described as parasitizing the larvae of land and fresh water arthropods, including mosquitoes, and are placed in the superfamily MERMITHOIDEA, family MERMITHIDAE (Chitwood, 1950). For a discussion of this group of parasites see Christie (1941), and Steinhaus (1949).

Mermithid parasites of the larvae of mosquitoes were first noted from this hemisphere by the author (1945a and 1945b). They were observed in fourth instar larvae of *Aedes vexans* and *Culex salinarius* from Delaware Co., Penna., and were seen to be invariably fatal to their hosts. Jenkins, in a paper before the Amer. Assoc. Econ. Ent. at Denver in December, 1950, reported a similar worm from the larvae of arctic and subarctic mosquitoes (northern Canada and Alaska). Drs. Jenkins and West kindly allowed me to see their manuscript on these worms. They present a thorough review of the literature, and plan extensive field and laboratory studies.

The writer is grateful to Miss Mary F. Allerton for the drawing of Fig. 1, and to Dr. Miriam I. Pennypacker for the sectioning of the larvae.

OBSERVATIONS

In August and September of 1944 fourth instar mosquito larvae were collected in tide and permanent pools at the edge of Cobb's Creek, south of Colwyn, Delaware Co., Penna. Some of these larvae were seen to have unusually whitish thoraces, and their movements were sluggish. Dissection showed them to be infected with larval mermithids. These worms were milky white, and those which emerged normally from their hosts ranged in length from 9 mm. to 18 mm., averaging 14.7 mm. Their genus and species as yet have not been determined.

In all, 217 larvae were collected from this area, with 90 harboring mermithids. The species of mosquito and number infected were as follows: 146 *Aedes vexans,* 76 infected; 53 *Culex salinarius,* 4 infected; and 18 *C. pipiens,* with 10 infected. The worms emerged through a hole in the side of the insect's thorax (Fig. 1). No effort was made to rear them. The only time when more than one worm emerged from a mosquito occurred when three small nematodes emerged from a single *C.*

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salinarius larva. The infected mosquitoes appeared unable to pupate, death invariably occurring very soon after the emergence of the worm from the grown larva. After the worm had emerged, the larva would give a few spasmodic jerks and ex-

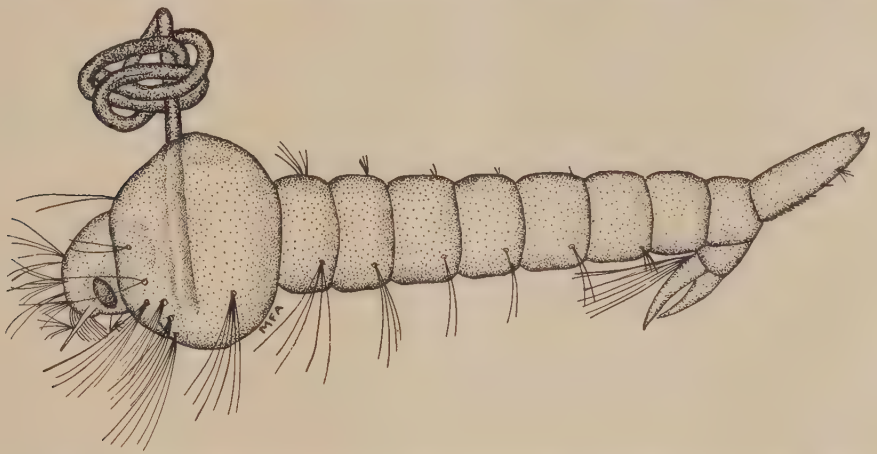

FIG. 1. Fourth instar larva of *Aedes vexans,* with a mermithid emerging from the side of the thorax.

pire. Unparasitized larvae pupated and gave rise to imagoes in the same dishes with dying, worm-infested larvae.

The sectioned thoraces showed that the worms lay coiled and twisted in the

FIG. 2. Cross-section of the thorax of a fourth instar larva of *Aedes vexans,* showing the relationship of the sections of the mermithid to the internal anatomy of the mosquito. W = mermithid.

haemocoel, just under the exoskeleton (Fig. 2). In following a worm from section to section it was seen to encircle completely the thoracic structures. A study of the sections did not reveal gross anatomical changes attributable to the presence of the

parasite. The death of the larvae shortly after the emergence of the worms suggests, however, that some damage may be attendant upon the worm's exit. No sections were made of the larvae which had lost their worms. No worm was seen to invade a larva's head or abdomen.

DISCUSSION

An estimation of the role of these mermithids in mosquito reduction must wait further information concerning their abundance and distribution. As no infected larva appears capable of survival to pupation, however, considerable potentiality for control seems indicated.

SUMMARY

1. Larval mermithid nematodes were found in fourth instar mosquito larvae from pools adjacent to Cobb's Creek at Colwyn, Delaware Co., Penna.

2. Seventy-six of 146 *Aedes vexans,* 4 of 53 *Culex salinarius,* and 10 of 18 *C. pipiens* were infected.

3. The worm completely prevented the mosquito's pupation, and the latter died soon after the worm's emergence from the insect's thorax.

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THE REPRODUCTIVE POTENTIAL OF FIVE SPECIES OF COCCIDIA OF THE CHICKEN AS DEMONSTRATED BY OOCYST PRODUCTION

STERLING BRACKETT AND ALEXANDER BLIZNICK
Stamford Laboratories, American Cyanamid Co., Stamford, Conn.

In an earlier paper we suggested that certain species of coccidia of chickens were better oocyst producers than others (Brackett and Bliznick, 1950). This observation was based on our studies of the number of oocysts produced following inoculations of relatively small numbers of oocysts in young birds. We even went so far as to rate several species according to what we thought was the relative oocyst productivity. It is now clear, from further studies, as well as from the helpful suggestions of others, that we were considering only a small part of all the factors involved in oocyst production. While the statement that some species are better producers than others is correct in general, the exact relationships between the species are not nearly so simple as we had originally thought. This report is an attempt to expand the observations on oocyst production with some additional experimental data.

The following factors, at least, affect the number of oocysts produced by a coccidial infection:

1. The inherent potential of each individual parasite to reproduce in a non-immune host.
2. The immunity or resistance developed by the host which may interfere with the normal reproductive potential of the parasites.
3. The "crowding" factor, the presence of which has been intimated by others (Tyzzer, *et al,* 1932, and personal communications from other investigators), and which will be considered in particular in this report. This factor may actually in part or in whole be explained by immunity, but we have not verified this as yet and can only offer certain tentative possible explanations of what it is (see discussion).
4. Competition with other species of coccidia or other infectious agents which also may or may not be part of the explanation of the "crowding" factor.
5. Nutrition of the host.
6. Strain differences of the host in susceptibility to coccidiosis.

There may, of course, be other factors we have overlooked. The studies reported here relate directly only to numbers 1 and 3 above.

EXPERIMENTAL

Species of Coccidia and Their Origin: Five species of coccidia were used in these studies. They are as follows:

E. tenella. A strain of this organism was obtained from the Zoological Division of the Bureau of Animal Industry, U. S. Department of Agriculture, through the courtesy of Dr. E. W. Price, in 1944. Since then it has been maintained in our

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laboratories by conventional methods of transfer with suitable precautions taken to keep it uncontaminated with other species.

E. necatrix. A strain of this species was obtained from Dr. E. H. Peterson, University of Washington, in April, 1949. It was free of *E. tenella* but contained small numbers of oocysts of both *E. acervulina* and *E. maxima.* The strain was "purified" of contaminants at intervals by merozoite transfer in the following manner. Fairly large numbers of oocysts (50–200 thousand) were given to a number of "clean" chickens. On the 5th day, merozoites were collected in intestinal scrapings. These were inoculated rectally into other clean birds. Two days later (7 days after the original oocyst inoculation) oocysts were collected from the ceca. The passage of merozoites from the intestine on the 5th day eliminates *E. tenella* and collection of oocysts only from the ceca eliminates the intestinal forms. It is usually necessary to make one further regular passage of oocysts to build up oocyst numbers

E. acervulina. This species was isolated from Peterson's material mentioned above by collecting only those oocysts being shed on the fourth day of the infection. Several passages in this way yielded a culture containing large numbers of oocysts which agreed with descriptions of the species (Tyzzer, 1929) as to measurements and produced characteristic lesions. Once the infection was pure, oocysts were collected on the 6th day when they are produced in much greater numbers.

E. maxima. This species was obtained from the Zoological Division of the Bureau of Animal Industry, U. S. Department of Agriculture, through the courtesy of Miss Marion Farr. This species was evidently originally obtained by single oocyst isolation. It has been maintained reasonably, but not completely, free from other species in our laboratories by careful handling.

E. brunetti. A strain which we are tentatively calling *E. brunetti* Levine, 1942, was isolated from chicken yard soil collected on a farm close to Stamford, Conn., in July, 1949. After building up numbers of oocysts by several passages, oocysts were collected from intestinal scrapings of lower intestine at times when they appeared to be ripe. Such material contained predominantly one species that resembled the descriptions of *E. brunetti* in size, although a few smaller oocysts, presumably *E. acervulina* and what appeared to be a pure culture of an organism resembling *E. brunetti* in oocyst size, life cycle and pathogenicity was obtained. This material has been offered to other investigators for confirmation of identification.

The methods used for infecting and for collecting and counting oocysts were essentially the same as described earlier (Brackett and Bliznick, 1949).

RESULTS

E. necatrix. Tyzzer *et al* (1932) called attention to the fact that better oocyst yields of this species were obtained with light infections than with heavy infections. Following this suggestion, we have always used relatively small inoculations (one to two thousand oocysts per bird) in our routine passage of this species with good results. Two preliminary tests to investigate this point quantitatively indicated quite clearly that increasing the size of the inoculum above a surprisingly low level did not result in a corresponding increase in oocyst production (Tables 1

TABLE 1.—*Daily oocyst production of mild infections with* E. necatrix *in chickens 2–3 weeks old (5 birds per group)*

Group	No. Oocysts Inoculated	Av. Number Oocysts (in millions) per Bird on Day Indicated								Approximate Oocyst Production per Oocyst Inoculated
		In Feces					In Ceca at end of 10th Day	Total	Av.	
		6	7	8	9	10				
1	200	<0.03*	0.58	1.56	3.64	1.30	1.56	8.64	10.12	50,000
2	200	<0.03	0.13	2.96	3.58	2.40	2.53	11.60		
3	2000	<0.03	0.09	1.50	0.97	0.97	1.17	4.70	4.75	2,500
4	2000	<0.03	0.62	0.42	1.88	0.97	0.91	4.80		

* i.e., none seen in counts made.

and 2). In fact, in the younger birds in table 1, fewer oocysts were produced following inoculation with 2000 oocysts than with 200 oocysts.

Following these preliminary experiments, a somewhat more detailed study of the relationship between the intensity of the infection and the resulting oocyst production was made in duplicate experiments (Table 3).

E. tenella. The oocyst production of light infections with this species was investigated rather extensively in connection with studies on various drugs (Brackett

TABLE 2.—*Oocyst production of mild infections with* E. necatrix *in young and older chickens (5 birds per group)*

Group	Age of Birds at Outset	Av. Number Oocysts (in millions) per Bird				Approximate Oocyst Production per Oocyst Inoculated
		No. Oocysts Inoculated	In Feces 7–9th Days	In Ceca on 9th Day	Total	Av.
1	9 Days	200	3.31	2.89	6.20	5.15	26,000
2	" "	200	3.42	0.69	4.11		
3	2 Months	200	1.56	0.26	1.82	2.51	12,500
4	" "	200	1.95	1.26	3.21		
5	" "	2000	10.53	1.36	11.89	9.52	5,000
6	" "	2000	4.68	2.47	7.15		

and Bliznick, 1949). Only mild infections were used (2000 oocysts per bird) since it was taken for granted that the formation of cecal cores in more severe infections would interfere with oocyst elimination if not also with oocyst production. These studies, which have been published, will be commented on in the discussion. For comparative purposes an experiment using graded infections of this species was run (Table 4).

E. brunetti. Studies on the oocyst production of this species consisted of two

TABLE 3.—*The oocyst production of graded infections with* E. necatrix *in chickens about 10 days old (5 birds per group in exp. 1 and 7 birds per group in exp. 2)*

Exp.	Group	No. Oocysts Given to Each Bird	Av. Number Oocysts* (in millions) per Bird	Approximate Oocyst Production per Oocyst Inoculated
1	1 & 2	50	2.93 (4.49, 1.37)	58,000
	3 & 4	250	4.99 (5.85, 4.14)	20,000
	5 & 6	1250	2.80 (2.32, 3.28)	2,000
	7 & 8	6250	6.72 (7.80, 5.65)	1,000
	9 & 10	35000	4.20 (5.46, 2.93)	100
2	1 & 2	50	0.77 (0.98, 0.56)	15,000
	3 & 4	250	2.66 (2.66, 2.66)	10,000
	5 & 6	1250	2.45 (1.82, 3.08)	2,000
	7 & 8	6250	2.38 (2.52, 2.24)	400
	9 & 10	35000**	0.50 (0.10, 0.97)	15

* Group averages given in parentheses. Principal figure is mean of group average.
** Three birds in each of Groups 9 and 10 died of an acute infection of *E. necatrix*; therefore, the counts for these groups are based on four birds each.

TABLE 4.—*The oocyst production of graded infections with* E. tenella *in chickens about 2 weeks old (7 birds per group)*

Group	No. Oocysts Given to Each Bird	Av. Number Oocysts* (in millions) per Bird	Approximate Oocyst Production Per Oocyst Inoculated
1 & 2	50	4.4 (4.4, 4.4)	80,000
3 & 4	250	15.1 (15.0, 15.2)	60,000
5 & 6	1,250	31.4 (27.1, 35.6)	25,000
7 & 8	6,250	62.6 (60.1, 65.0)	10,000
9* & 10*	20,000	34.9 (34.3, 35.4)	1,750
11* & 12*	40,000	47.9 (52.8, 42.9)	1,200

* Birds died from cecal coccidiosis on the 5th and 6th days as follows: 2 birds of group 9; 3 of group 10; 4 of group 11; and 5 of group 12.

experiments with graded doses of inoculated oocysts. In the first experiment, oocyst counts were made daily while in the second test the fecal material for the 4 peak days was pooled for counting. Since nothing is available in the literature on the oocyst production of this species, we are giving our data in somewhat greater detail.

TABLE 5.—*Daily oocyst production of graded infections with* E. brunetti *in young chickens 2–3 weeks old (7 birds per group)*

Group	No. Oocysts Inoculated	Av. Number Oocysts (in millions) per Bird on Day Indicated						Mean of Group Averages	Approximate Oocyst Production per Oocyst Inoculated
		In Feces				In Intestines At End of 9th Day	Total		
		6	7	8	9				
1	50	1.13	2.37	2.10	0.81	....	6.41	5.39	108,000
2	50	0.14	1.30	1.38	1.42	0.13	4.37		
3	250	3.22	12.83	6.13	4.75	....	16.93	21.04	84,000
4	250	2.80	15.80	3.50	1.98	1.06	25.14		
5	1,250	5.67	15.90	1.67	3.01	....	26.25	29.50	24,000
6	1,250	6.68	15.80	6.07	4.00	0.20	32.75		
7*	6,250	15.40	17.30	4.32	3.57	....	40.59	43.07	7,000
8*	6,250	20.20	15.05	6.60	2.82	0.88	45.55		
9*	20,000	12.70	11.15	3.77	1.43	....	29.05	34.01	1,700
10*	20,000	17.75	10.80	6.65	3.62	0.15	38.97		
11*	40,000	7.66	5.63	2.20	1.17	....	16.66	21.58	500
12	40,000	8.00	11.05	4.42	2.91	0.13	26.51		

* One bird died in each of groups 7, 8, 10, and 11, and 2 died in group 9.

E. acervulina and *E. maxima.* Many of the data available have already been published (Brackett and Bliznick,1950). Two bits of additional information not previously recorded, but which will be referred to in a later section of the paper, are as follows. Ten birds, about 2 months old, were infected with 500,000 oocysts of *E. acervulina.* The average oocyst production of these birds during the sixth day of the infection was 432 million (about 1,000 oocysts per oocyst inoculated). Ten birds about 2 months old were infected with 650,000 oocysts of *E. maxima.*

TABLE 6.—*The oocyst production of graded infections with* E. brunetti *in young chickens 2–3 weeks old (14 birds per group)*

Group	No. Oocysts Inoculated	Av. Number Oocysts (in millions) per Bird	Approximate Oocyst Production Per Oocyst Inoculated
1	50	19.3	400,000
2	250	36.9	150,000
3	1,250	32.3	26,000
4	6,250	44.2	7,000
5*	20,000	16.6	800
6*	40,000	17.1	400

* One bird died in group 5 and 2 birds died in group 6.

During the sixth day of their infection, the average output of oocysts per bird was 9.3 million.

DISCUSSION

The number of oocysts produced by each oocyst inoculated in the experiments referred to in this paper and in previous reports (Brackett and Bliznick, 1950) is of interest in indicating the approximate reproductive potential for the different species under the conditions observed. The highest figure obtained for each species is given, for summary purposes, in table 7.

TABLE 7.—*Summary of the approximate maximum reproductive potential of various species of* Eimeria

Species	Maximum Number of Oocysts Produced per Oocyst Inoculated
E. brunetti	400,000
E. tenella	400,000
E. acervulina	72,000
E. necatrix	58,000
E. maxima	12,000

These figures cannot be taken too seriously since there was considerable variation from experiment to experiment. However, they probably do indicate, in a rough way, the relationship between these species in respect to the multiplicative potentials under optium conditions, i.e., light infections in non-immune birds. It was in this respect that we suggested, in an earlier paper (Brackett and Bliznick, 1949) that certain species were better oocyst producers than others. The most remarkable feature of the data presented in this paper, however, it that, at least with some of the species, there is a marked reduction in this reproductive potential as the size of the original inoculum is increased. It is almost as if the parasites became too crowded to reproduce to the maximum extent.

TABLE 8.—*The maximum total oocyst productive potential of various species of* Eimeria *of chickens**

Species	Inoculum	Av. Number of Oocysts Produced per Bird in Group Selected
E. acervulina	500,000	432,000,000
E. tenella	6,250	65,000,000
E. brunetti	6,250	53,000,000
E. maxima	10,000	36,000,000
E. necatrix	2,000	12,000,000

* In all cases the oocyst production studies were made on groups of 5–7 birds. Frequently duplicate groups were run. In some of the tables in an earlier section, the results of duplicate groups were averaged to give a single figure. The figures in this table represent individual groups, whichever gave the highest count.

From a practical aspect, the maxium total number of oocysts which an infection can produce under any conditions is probably at least as important as the maximum reproductive potential of the species under certain limited conditions. From all our data we have selected the experimental group producing the largest number of oocysts of each species to illustrate this point (Table 8). In this respect, *E. acervulina* would seem to be the best producer of oocysts of all the species studied, with *E. necatrix* the poorest.

Information of this sort would be of value if it helped to explain the nature and occurrence (epidemiology) of coccidiosis in the field. It is well known that resistance to coccidiosis develops quite readily. This developing resistance undoubtedly

restricts the number of oocysts that are produced. Without knowing the extent to which immunity influences the oocyst production of the different species it is difficult to generalize as to what might be happening under field conditions. The following implications are suggested but must be taken with considerable reservations:

1. Starting with a comparatively clean environment with only a few viable oocysts available, *E. tenella* and *E. brunetti* should increase most rapidly because of the high reproductive potential in light infections (Table 7).

2. With somewhat greater numbers of oocysts available at the outset, or due to increase by passage through chickens, *E. acervulina* contamination may increase most rapidly because its multiplication is relatively unhampered by the so-called "crowding" effect (Table 8).

3. The relatively unlimited oocyst production capacity, regardless of inoculum size, of *E. acervulina,* compared with other species, may help explain why it is this species and not the others that is occasionally incriminated in outbreaks of coccidiosis shortly after pullets are transferred to laying houses. The non-immune birds coming in from dry ranges where oocyst sporulation is impossible, build up *E. acervulina* so fast under the crowded, moist conditions in the laying house, that pathogenic concentrations occur before all the birds become immune. With the other species, in which oocyst numbers build up more slowly, the birds evidently become immune before dangerous concentrations occur.

4. The very low oocyst potential of *E. necatrix,* in both oocysts per oocyst and total oocysts, may account at least in part for its generally delayed appearance in a flock as compared with *E. tenella.*

5. The combination of low pathogenicity and poor oocyst production most likely accounts for the rarety of outbreaks with *E. maxima.*

We can offer no explanation at present for the so-called "crowding" effect of increasing infections except possibly in *E. tenella* where there may be extensive tissue damage and cecal core formation which may interfere with the infection. Several possibilities suggest themselves, however. It is conceivable that the parasites may produce some sort of a toxin which interferes with their own development. Heavier infections would produce more of this material, thus causing greater interference. What seems to us to be a more likely possibility, but one difficult to prove, is that the first generation parasites produce antigen, which works quickly enough to cause a host reaction which interferes with the oocyst production of later generations, the heavier the initial oocyst infection the greater the amount of antigen during the first generation. Since the oocyst producing generation (or generations) appear only a few days after the first generation which develops from the sporozoites, a very rapid immune response must be postulated. The speed with which resistance appears in these infections is not clearly known. It is of interest to note that the species with the shortest life cycle (*E. acervulina*) shows the least effect of "crowding" while species with the longest life cycle (*E. necatrix*) shows the greatest effect (Table 8).

SUMMARY AND CONCLUSIONS

1. The oocyst production of the following five species of coccidia of the chicken was studied in non-immune birds. *Eimeria tenella, E. necatrix, E. acervulina, E.*

brunetti, and *E. maxima.* The number of oocysts produced per oocyst inoculated varied from species to species.

2. With very mild initial infections (about 50 oocysts per bird) *E. tenella* and *E. brunetti* were about equally productive, yielding several hundred thousand oocysts for each oocyst inoculated. The other three species were progressively poorer oocyst producers in the following descending order: *E. acervulina, E. necatrix,* and *E. maxima. E. maxima* produced less than a tenth as many oocysts as the two most prolific species.

3. More severe initial infections did not necessarily yield progressively larger numbers of oocysts. This relationship varied from species to species. The greatest number of oocysts produced per bird by the different species was approximately as follows (in millions of oocysts): *E. acervulina,* 430; *E. tenella,* 65; *E. brunetti,* 55; *E. maxima,* 36; and *E. necatrix,* 12.

4. The number of oocysts produced under varying conditions in initial and in subsequent exposures is undoubtedly an important factor in the epidemiology of the different species and helps to explain the relative economic importance of each.

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THE MALE OF *DERMACENTOR DISSIMILIS* COOLEY (ACARINA: IXODIDAE)[1]

GLEN M. KOHLS[2] AND HERBERT T. DALMAT[3]

Dermacentor dissimilis Cooley was described (1947) from 6 females and 2 nymphs collected from horses in Chiapas, Mexico, February 17, 1945. Although no further records of the species have been reported to date it may be noted that Bequaert's (1938) record of "*Dermacentor nigrolineatus* Packard" off horse, Pacayal, Guatemala, 1931, refers to *D. dissimilis* as evidenced by a female in the Rocky Mountain Laboratory collection bearing the same collection data.

Males of *D. dissimilis* were first discovered in a collection totaling 6 males and 13 females of this species from horses, Zongolica, Veracruz, Mexico, July 2, 1948, which was submitted to the senior author for identification by Dr. C. Ortiz Mariotte. Subsequently 40 lots of this tick, including numerous males and immature stages, were collected in Guatemala by the junior author. All were from horses and cattle at San Pedro Yepocapa and Acatenango, Department of Chimaltenango, elevation 2900 feet to 5700 feet, and were collected during the periods of July to November 1950 and February to July 1951. In several instances larvae, nymphs, and adults were found on the same host at the same time, thus confirming Cooley's earlier surmise that *D. dissimilis* is a one-host tick.

Dermacentor dissimilis Cooley
Male, fig. 1 and pl. 1, A

Length from humeral angles to caudal margin from 2.15[4] to 2.50 (13 specimens measured); width from about 1.56 to 1.85. Shape suboval, widest just anterior to the spiracular plates. Base color brown and with grey ornamentation varying in distinctness and extent in individual specimens. The ornamentation is usually restricted to the scutum but sometimes is faintly visible on the basis capituli dorsally and on the legs. Body usually a little contracted in the vicinity of the spiracular plates.

Capitulum: Length from tips of palpi to tips of cornua from 0.47 to 0.58; width of basis from 0.35 to 0.44. Basis wider than long; lateral margins parallel, posterior margin between the cornua concave; punctations and hairs absent. Cornua subacute and about as long as wide. Palpi short, broad and rounded apically, posterodorsal angle on article 2 only moderately developed. In ventral view, basis is convex, posterior margin broadly rounded. Palpal article 3 with a mild retrograde spur.

Hypostome: Shape as figured. Dentition remarkably variable; in 20 of 46 male hypostomes studied the dentition was 3/3, in 6 it was 3/4 or 4/3, in 7 it was 3½/3½, and in 13 the dentition was 4/4. Length about 0.27.

Scutum: Cervical grooves represented by inconspicuous pits or short shallow depressions in the anterior cervical region. Lateral grooves absent. Punctations quite variable in size, number, and distribution; usually larger and more numerous in the lateral and humeral areas. Hairs small, sparsely distributed, but more abundant posteriorly and laterally. Eyes distinct in most specimens seen but, as in the female, they are obsolescent in some and apparently absent in others. Festoons 11 in number.

Legs: Coxa I bifid, internal spur wider and a little shorter. Coxae II, III, and IV with external spurs only. All coxae with a few fine hairs. Mild spurs on trochanters I, II, and III

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[1] From the Federal Security Agency, Public Health Service, National Institutes of Health, National Microbiological Institute.

[2] Rocky Mountain Laboratory, Hamilton, Montana.

[3] Laboratory of Tropical Diseases, Bethesda, Maryland.

[4] All measurements are in millimeters.

PLATE 1. *Dermacentor dissimilis* Cooley. A. Male. B. Female. (N. J. Kramis, phot.)

ventrally; a moderate retrograde spur on trochanter I dorsally. Tarsus I about 0.44 in length and with an apical ventral spur; metatarsus about 0.34. Tarsus and metatarsus IV each about 0.44 in length; the former with an apical and a subapical ventral spur.

Spiracular plate: Shape as figured. Greatest dimension about 0.37. Goblets few.

Genital aperture: Situated at the level of the intervals between coxae I and II.

Selected as the neallotype is one of 13 males from horse, Finca Concepcion, Acatenango, Chimaltenango, Guatemala, October 30, 1950, H. T. Dalmat coll. (RML No. 28460.) Deposited in the collection of the Rocky Mountain Laboratory.

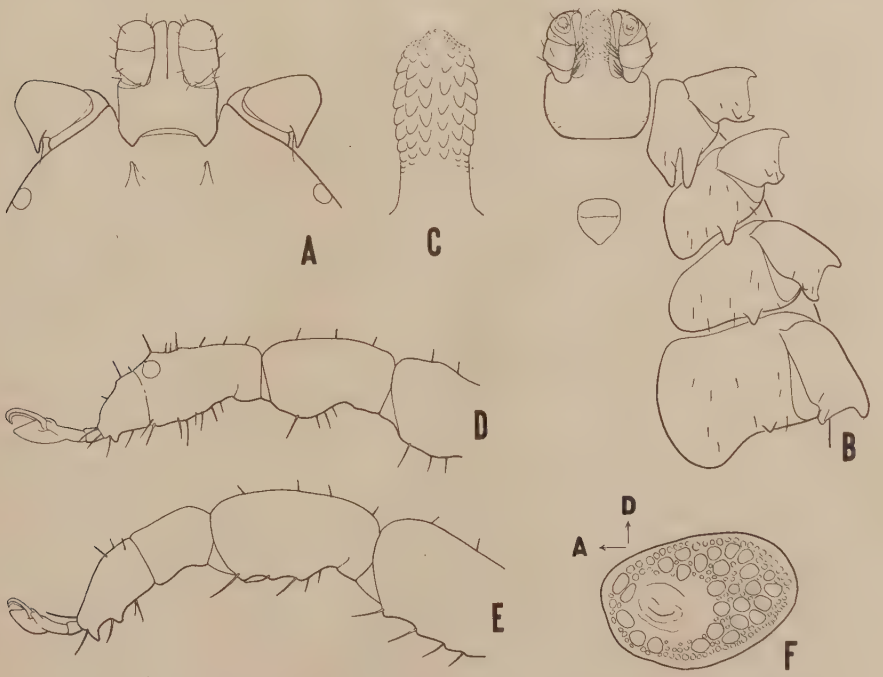

FIG. 1. *Dermacentor dissimilis* Cooley. Male. A. Capitulum and anterior portion of scutum, dorsum. B. Capitulum and coxae, venter. C. Hypostome. D. Metatarsus and tarsus, leg I. E. Metatarsus and tarsus, leg IV. F. Spiracular plate (a = anterior; d = dorsal).

REMARKS

The male of *D. dissimilis* can be readily separated from the male of *D. albipictus,* which it resembles superficially, by its shape and by the absence of internal spurs on coxae II and III. *D. albipictus* is definitely more elongate and internal spurs are present on these coxae. *D. albipictus* is a larger species, and the dentition of the hypostome is 3/3 in all males and females of this species that the senior author has examined.

In the original description of the species Cooley (1947) called attention to the variation in the dentition of the hypostomes of the 6 females available to him for study. He observed that in one specimen the formula was 4/3 and that in the other 5 it was 4/4 with some of the denticles out of line in the files. Examination of 101 females in the present series revealed that in 95 the formula was 4/4, in 3 it was 3/4 or 4/3, and in 3 others is was 3½/3½. The dentition in the nymphs appears to be consistently 3/3, as compared with 2/2 in *D. albipictus.*

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TWO NEW VENEZUELAN CHIGGERS (ACARINA: TROMBICULIDAE)

James M. Brennan
Federal Security Agency, Public Health Service
National Institutes of Health, National Microbiological Institute*

Two new chiggers were discovered among ectoparasites collected in northern Venezuela in the course of a plague survey under the direction of the United States Naval Medical School during the summer of 1950. One of these represents a new genus and species, and the other, a new species of *Acomatacarus,* both of which are described below.

Anomalaspis, new genus

Trombiculid larvae of the subfamily Walchiinae. Eyes present. Cheliceral blade with tricuspid cap. Scutum with 3 setae, the anterolaterals and anteromedian; the posterolateral setae apparently displaced onto the dorsum. Sensillae flagelliform.

Genotype: Anomalaspis ambiguus n. sp.

The generic name has been compounded from the Greek meaning "unusual shield." *Anomalaspis* is unique among all other genera of the Walchiinae by virtue of the flagelliform sensillae.

Anomalaspis ambiguus n. sp.
(fig. 1)

Body: Length and width of holotype, slightly engorged, 260 by 156 microns. Striae conspicuous. Eyes 2/2, on an ocular plate. Anus located at about the level of the fifth row of ventral setae.

Gnathosoma: Cheliceral bases, capitular sternum and a basal plate on palpal femur punctate. Cheliceral blade with a tricuspid cap. Palpal setae as follows: coxal with many long branches, femoral with few branches, genual with 2 branches, dorsal tibial with about 5 branches, lateral tibial with 2 branches, ventral tibial with 5 or 6 long branches. Palpal claw apparently trifurcate. Palpal tarsus with a spur, long subterminala, and 4 branched setae. Galeal seta nude.

Scutum: About as long as broad, with numerous punctae, the posterior margin broadly rounded and posterolateral angles absent. Scutal setae stout, with few short barbs. Only the anterolateral setae and anteromedian seta are on the scutum, the posterolateral setae presumably are displaced onto the dorsum and are regarded to be the paired setae, each of which is located at the posteroproximal margin of the ocular plate (see under description of setae). Sensillae branched on apical half. Sensillary bases widely separated and very near the anterior margin. Scutal measurements of holotype: AW-49, PW-72, SB-29, ASB-20, PSB-41, AP-29, AM-21, AL-18, PL-24, S- (broken in remounting).

Legs: All segments of all legs punctate. Setae distributed as follows: leg I coxa, trochanter and basifemur each with a branched seta: telofemur with 5 branched setae; genu with 4 branched setae, 2 genualae and a microgenuala; tibia with 8 branched setae, 2 tibialae (one of which is spur-like, the other more slender) and a microtibiala; tarsus with about 18 branched setae, a spur, a short parasubterminala, a subterminala, and a pretarsala. Leg II coxa and trochanter each with a branched seta; femur with 6 branched setae; genu with 3 branched setae and a genuala; tibia with 6 branched setae and 2 tibialae; tarsus with about 14 branched setae, a spur and a pretarsala. The microspur usually present on tarsi I and II of trombiculid larvae is absent. Spurs I and II are rather short and about equal in form and size. Leg III coxa and trochanter each with one branched seta; femur with 4 branched setae and 1 apparently nude seta; genu with 3 branched setae and a genuala; tibia with 6 branched setae and a tibiala; tarsus with about 12 branched setae. All tarsi terminated by 2 lateral claws with a longer claw-like empodium between them.

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* Rocky Mountain Laboratory, Hamilton, Montana.

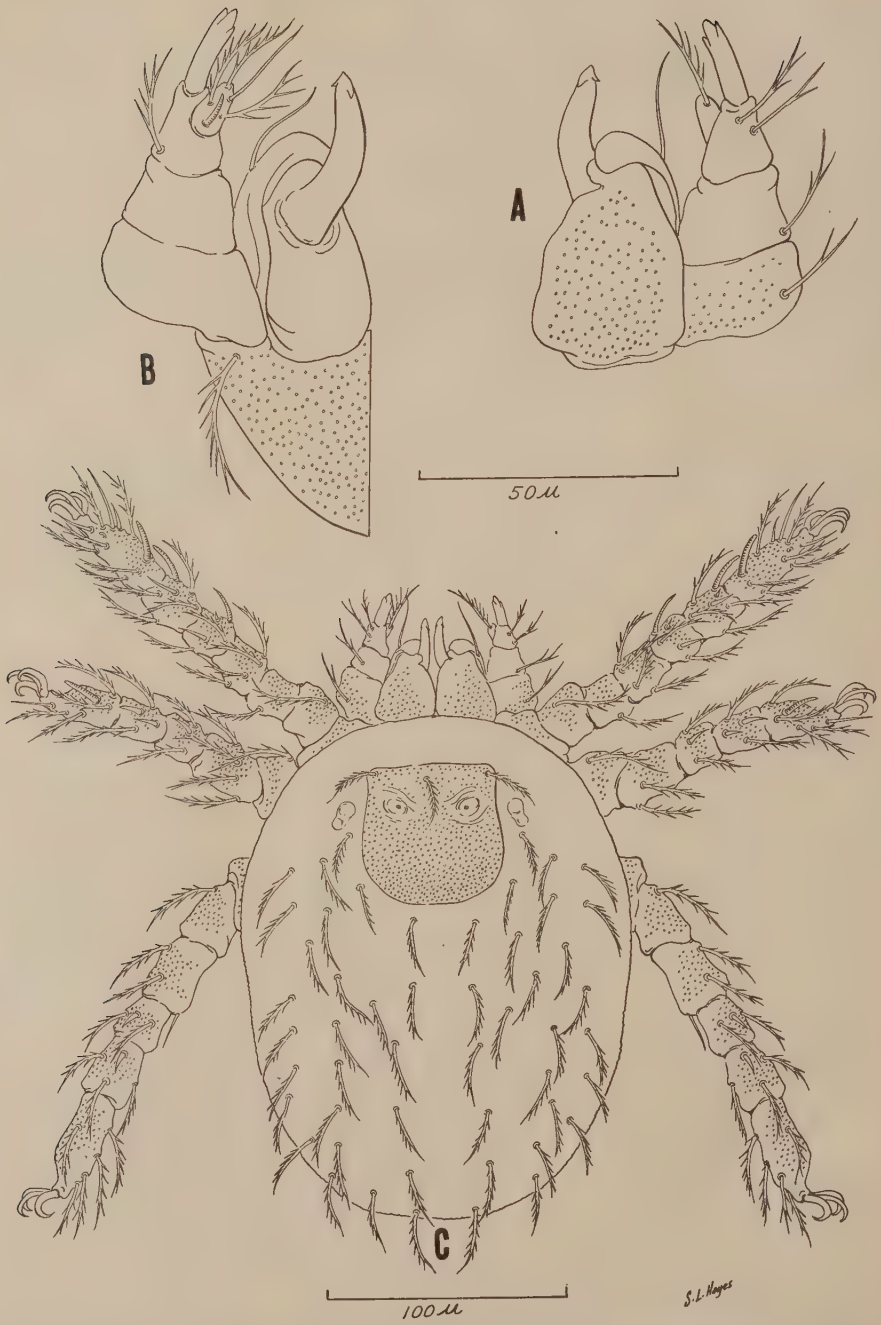

FIG. 1. *Anomalaspis ambiguus* n. sp. A. dorsal aspect of left half of gnathosoma; B. ventral aspect of left half of gnathosoma; C. dorsal aspect of holotype.

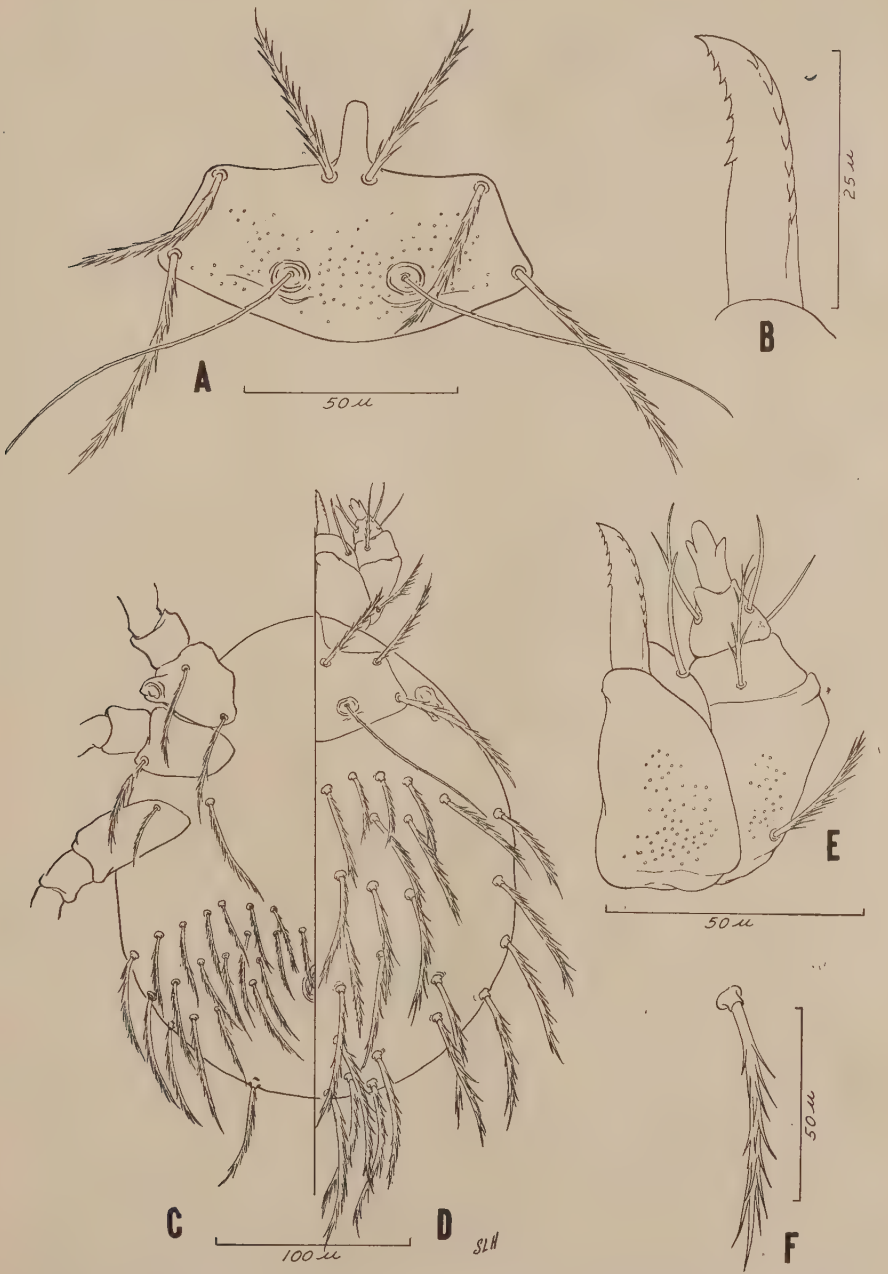

FIG. 2. *Acomatacarus tuberculoris* n. sp. A. scutum; B. cheliceral blade; C. venter; D. dorsum; E. dorsal view of right half of gnathosoma, palpal tarsus omitted; F. a dorsal seta and its tubercular base.

Setae: Dorsal setae (humerals excluded) 40, similar to the scutal setae, small with short barbs, approximately of uniform length from anterior to posterior rows and arranged irregularly as figured. On each side, posterolateral to the scutum is a group of 6 setae, 1 of which is regarded as the displaced posterolateral seta of the scutum and the remainder as humerals. There are 2 setae in this group of 6, either one of which, or both of which, might be displaced posterolaterals; however, for convenience, the writer has arbitrarily selected the seta as designated under the description of the scutum. Ventral setae 2–2 (sternals) plus about 50, those posterior to the anus the longer and similar to the dorsal setae in form. Both pairs of sternals are about equal in length.

Type data: Holotype, R.M.L. No. 28432, larva, from *Heteromys anomalus anomalus* (spiny pocket mouse), Campamento Rafael Rangel, Sierra Maestra, Aragua, Venezuela, summer of 1950, collected by Dr. Ernst Schwarz. Deposited in the collection of the Rocky Mountain Laboratory.

Unfortunately only one specimen of this interesting genus and species was available for description.

Acomatacarus tuberculariis n. sp.
(fig. 2)

Body: Striae fine, not prominent. Eyes 2/2, on an ocular plate. Length and width of holotype, somewhat engorged, 268 by 171 microns. Anus located at level of third row of ventral setae.

Gnathosoma: Cheliceral bases, capitular sternum and femoral and genual plates of the palpi punctate. Blade of chelicera with a row of 5 to 8 dorsal teeth increasing progressively in size from the tip and a row of about as many ventral teeth, sharp-pointed and widely separated, giving a serrate appearance to the margin. Palpal setae as follows: coxal rather short with very long branches; femoral and genual branched, sometimes serrate in appearance; dorsal tibial with short branches; lateral and ventral tibial nude. Palpal claw trifurcate. Palpal tarsus with 7 branched setae and a long spur. Galeal seta with short appressed branches.

Scutum: Roughly rectangular, much wider than long, with rounded corners, shallow convex posterior margin, and few scattered punctae. Sensillary bases well separated and at about the level of the posterolateral setae. Sensillae long, flagelliform, with few branches and the suggestion of minute barbs basally. Scutal setae heavily barbed. Length of anteromedian projection, 18 microns. Scutal measurements of holotype: AW-63, PW-81, SB-30, ASB-31, PSB-15, AP-24, AM-50, AL-51, PL-69, S-94.

Legs: All segments of all legs punctate. Setae distributed as follows: leg I coxa with 2 branched setae; trochanter with 1 branched seta; femur with 5 branched setae; genu with 4 branched setae, 2 genualae and a microgenuala; tibia with 8 branched setae, 2 tibialae and a microtibiala; tarsus with about 18 branched setae, a spur, a microspur, a large parasubterminala with a few branches, a subterminala, and a small pretarsala. Leg II coxa and trochanter each with a branched seta; femur with 5 branched setae; genu with 4 branched setae, a genuala and a microgenuala; tibia with 6 branched setae and 2 tibialae; tarsus with about 15 branched setae, a spur, a microspur and a small pretarsala. Leg III coxa and trochanter each with a branched seta; femur with 4 branched setae; genu with 4 branched setae; tibia with 6 branched setae and a tibiala; tarsus with about 12 branched setae and a mastitarsala. Tarsi of all legs terminated by a pair of claws with a longer and more slender claw-like empodium between. The unspecialized setae of the legs are variable in form and length.

Setae: Dorsal setae about 42, similar in form to the scutal setae and arising from small tubercles, in irregular rows arranged approximately 11–6–7–6–6–4–2, the humerals not distinguishable from those of the first row. The setae increase in length from anterior to posterior rows, and in the first row the medial setae are much shorter than those at the ends. Ventral setae 2–2 (sternals) plus about 40, those posterior to the anus larger and similar to the dorsal setae. Setae absent between coxae II and III.

Type data: Holotype and 18 paratypes, R.M.L. No. 28432, from *Heteromys a. anomalus* (spiny pocket mouse); Campamento Rafael Rangel, Sierra Maestra, Aragua, Venezuela; summer 1950, Dr. E. Schwarz, collector.

Holotype and some paratypes deposited in the collection of the Rocky Mountain Laboratory. Other paratypes distributed to the United States National Museum, the United States Naval Medical School, the British Museum (Natural History), the South Australian Museum, Adelaide, and the University of Kansas.

Diagnosis: A. tuberculariis is readily separated from other species of the genus in the Western Hemisphere by the small tubercles from which the dorsal and posterior ventral setae of the body arise.

The figures for the above descriptions were prepared by Sherman L. Hayes, staff artist of the Rocky Mountain Laboratory.

A NEW SPECIES OF *ISOSPORA, I. XANTUSIAE,* FROM SOUTHERN CALIFORNIA LIZARDS*

YOST U. AMREIN[1]

Department of Zoology, University of California, Los Angeles[2]

A hitherto undescribed species of *Isospora* has been discovered in several specimens of the Yucca Night Lizard, *Xantusia vigilis,* collected in the Mojave Desert, and in the Granite Night Lizard, *Xantusia henshawi,* collected in the Mt. San Jacinto region. The coccidian occurs in both species of *Xantusia* and parasitizes the cytoplasm of the epithelial cells lining the small intestine. Diagnosis was made from sectioned preparations stained with Delafield's haematoxylin; sporulation was observed in oocysts kept in a 5% dichromate solution.

Isospora xantusiae n. sp.

(Figs. 1–4)

Specific Diagnosis: Intracellular stages in cytoplasm of epithelial cells of small intestine. Schizonts spherical, 8 to 9.5 micra in diameter, 6 to 8 banana-shaped merozoites each 1.25 by 5.5 micra. Macrogametocytes round, 9 to 11 micra in diameter; macrogametes average 18.5 micra in diameter. Microgametocytes 12.5 to 15.5 micra in diameter with large number of comma-shaped microgametes. Oocysts free in gut lumen, spherical to subspherical in shape, without micropyle, 25 to 27 micra in longer axis. Sporulation time 12 to 24 hours, giving rise to two sporocysts, 10 by 15 micra, with a knob at one end, enclosing four spindle-shaped sporozoites. No oocystic residual body, but voluminous granular residual mass in sporocysts.

Hosts: Xantusia vigilis Baird.

Xantusia henshawi Stejneger.

Locality: Antelope Valley, Los Angeles County, Southern California. San Jacinto Mountain foothills, Riverside County, Southern California.

Holotype: Department of Zoology, University of California, Los Angeles, California.

DESCRIPTION

The oocysts are found in the intestinal contents in from few to immense numbers and are spherical to subspherical in shape (see Figs. 1–3). The colorless cyst-wall is about 1.5 to 2 micra thick and appears to be made up of but two membranes. There is no oocystic micropyle. The form index (width : length) fluctuates between 0.9 and 1.0. Approximately two-thirds of the oocysts encountered upon examination of fresh material already possess two well-defined spherical sporoblasts while the rest show but one large spherical or ovoidal granular mass, varying in size from 14 to 16 micra.

When placed in 5% dichromate solution in a moist chamber at room temperature, almost all oocysts will show the two sporoblasts within twelve to twenty-four hours. Twenty-four to forty-eight hours after removal from the intestinal environment, sporulation is completed for all the cysts that will develop.

The oval sporocysts measure on the average 9.9 by 14.8 micra and have a definite knob and refractile globule at one end. The sporocyst wall is double-contoured

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[2] Present address: Department of Zoology, Pomona College, Claremont, California.

and encloses four faintly discernible sporozoites twisted about one another and a great many globular masses which obscure from view the banana-shaped sporozoites. There is no single formed residual sporocystic mass, the granular aggregations of protoplasm being scattered throughout the spores. While a relatively voluminous residual mass is present in the sporocysts, none whatsoever is found in the oocyst.

The intracellular stages of this coccidian are found in the epithelium of the small intestine only (Fig. 4). Although these stages are encountered throughout the entire length, the heaviest tissue invasion appears to be in the lower half of the small intestine. The tissue forms of this parasite are embedded superficially between the host-cell nucleus and the striated border. In sections of parasitized intestine, all the stages of the schizogonous and the gamogamous cycle can be seen in the epithelial layer of one or two villi.

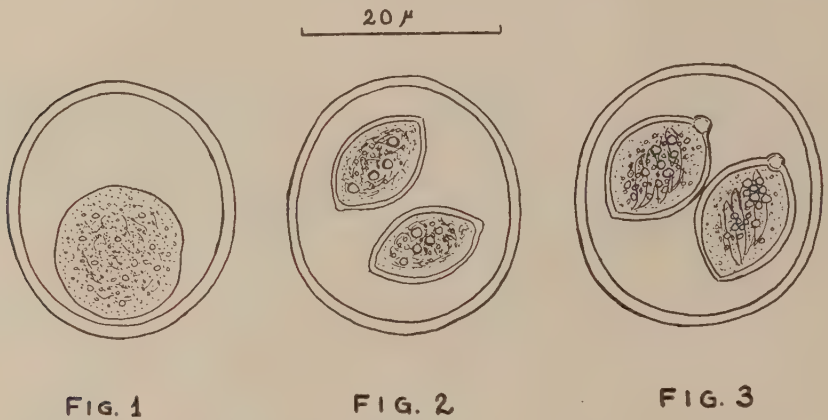

FIG. 1. Oocyst with single granular mass.
FIG. 2. Oocyst with two immature sporocysts.
FIG. 3. Oocyst with two fully-formed sporocysts, each containing four sporozoites and a large number of granular inclusions.

The earliest stage, a trophozoite lying close to the periphery of the host cell, appears as a round mass of cytoplasm with a distinct nucleus. It measures 4.5 to 5 micra in diameters and exhibits a faint halo. Spherical schizonts, 8 to 9.5 micra in diameter, occupy a position about one-third the distance from the host cell border to the nucleus. Various numbers of nuclear fragments are seen in the younger schizonts. Six to eight banana-shaped merozoites, 1.25 by 5.5 micra, are enclosed in the mature schizont which, by this time, assumes a slightly oblong shape and reaches a size of 7.5 by 9 micra.

The macrogametocytes appear as rounded pieces of dense cytoplasm with deeply-stained nuclei, are usually surrounded by a chromophilic membrane, and measure from 9 to 11 micra in diameter. Mature macrogametes are the largest intracellular forms encountered and ordinarily lie close to the periphery of the host cell. The cytoplasm of the ball-like gamete, measuring on the average 18.5 micra in diameter, appears alveolar with a well-staining nucleus, the latter about 3 micra in diameter.

Microgametocytes first appear as oval bodies, 7.5 by 12 micra, with numerous nuclear fragments. When mature, the microgametocytes measure on the average 12.5 by 15.5 micra and contain a large number of comma-shaped, deeply-staining microgametes, each about 0.5 by 3 micra in size.

FIG. 4. Photomicrograph of sectioned small intestine of *Xantusia vigilis* showing intracellular stages of *Isospora xantusiae* in epithelial cells.

DISCUSSION

Nineteen species of *Isospora* are known presently among terrestrial cold-blooded vertebrates. In addition to these described species, four unnamed *Isospora* have been reported in the literature. Among REPTILIA, all the *Isospora* recorded occur in squamate hosts, none having been found in CHELONIA or in LORICATA. Seven *Isospora* species are known from ophidian hosts, while five have been recovered from SAURIA.

With the exception of *I. lieberkühni* (Labbé, 1894) which parasitizes the kidney of the frog, and possibly *I.* sp. in *Agama colonorum* (Wenyon, 1926), all the *Iso-*

spora known from AMPHIBIA and REPTILIA undergo their schizogonous cycle in the host's intestinal epithelium.

A comparison of the new species with known *Isospora* shows *I. xantusiae* to have certain affinities with the following saurian *Isospora: I. calotesi* Bhatia, 1938; *I. camillerii* Hagenmüller, 1898; *I. mesnili* Sergent, 1902; and *I. knowlesi* Ray and Das Gupta, 1937.

Although size and morphology of sporocysts relate the present species closely to *I. calotesi,* the latter, according to Chakravarty and Kar (1946), possesses an oocystic micropyle and only a small spherical residual mass in the sporocyst. The xantusiid *Isospora* differs from *I. camillerii* in oocyst and sporocyst size and shape, and above all in sporulation time which is two weeks for *I. camillerii.* Both *I. mesnili* and *I. knowlesi,* although having sporocysts with knobs, differ markedly from the currently investigated species in that they parasitize the nuclei of the epithelial cells. There is thus no previously reported *Isospora* fitting the characteristics of the xantusiid species. Since this *Isospora* occurs fairly commonly in both *X. vigilis* and *X. henshawi,* the name *I. xantusiae* is proposed for it.

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HELMINTHS FROM THE ROUND-TAILED MUSKRAT, *NEOFIBER ALLENI NIGRESCENS* HOWELL, WITH DESCRIPTIONS OF TWO NEW SPECIES

ROBERT RAUSCH[1]

The helminths parasitic in the round-tailed muskrat, a microtine rodent of very restricted geographical range, have not been investigated. Two specimens of this mammal, collected in Putnam County, Florida, were included among a collection of microtine rodents made available to the writer by Mr. W. B. Quay, Museum of Zoology, University of Michigan. Appreciation is expressed here for the opportunity to examine this valuable material.

Both the animals were infected by helminths, of which four species were represented. One harbored two species of cestodes and one species of trematode in the intestinal tract; the other showed a heavy cysticercus infection of the liver. Two species, a trematode and a cestode, are described as new. The remaining two species constitute new host records.

Several hundred small trematodes of the genus *Quinqueserialis* Skvortsov, 1935, were found in the cecum of one of the rodents. These specimens clearly represent an undescribed species:

Quinqueserialis floridensis n. sp.

(Figs. 1–3)

Diagnosis: Body oval, slightly attenuated anteriorly, with concave ventral surface; 1.2 to 1.7 mm. long by 700 to 900 μ in greatest width. Cuticular spines not observed. Ventral surface with five rows of glands; mesal row has 16 glands; paramesal rows have 16 glands; lateral rows have 13 or 14 glands. Oral sucker from 140 to 165 μ in diameter. Esophagus typical for genus. Sinuous intestinal crura pass mediad of vitellaria and testes, and terminate just below margins of latter. Excretory pore median, situated at level just posterior to ends of intestinal ceca. Lobed testes immediately posterior to vitellaria; testes measure 140 to 180 μ in greatest length. Long axis of testes transverse, oblique, or longitudinal. Seminal vesicle well developed; convolutions numerous at posterior end of cirrus sac. Cirrus sac, 310 to 430 μ long by about 75 μ in greatest width, extends from genital pore near bifurcation of intestinal ceca posteriorly to point at level of most anterior loop of uterus. Cirrus provided with numerous conical spines. Coarsely lobed ovary intercecal, situated at same level as testes. Ovary size about half that of testes. Well-developed Mehlis' gland pre-ovarian. Uterus has seven, sometimes eight, transverse loops which extend laterally beyond intestinal ceca and vitellaria, nearly reaching body margins. Metraterm strongly developed, averaging half the length of cirrus sac. Vitellaria consist of two lateral groups of 12 to 15 follicles, compactly arranged. Vitellaria situated extracecally just anterior to testes, which they approximate in size, and almost wholly posterior to transverse uterine loops. Vitelline ducts strongly developed. Ovoid eggs from 14 to 16 μ long by 8 to 10 μ wide. Polar filament not observed.

Host: Neofiber alleni nigrescens Howell.

Habitat: Cecum of host.

Locality: Putnam County, Florida.

Type: A slide, No. 47587, containing whole mounts of paratype material, has been deposited in the Helminthological Collection of the U. S. National Museum.

Quinqueserialis floridensis, the smallest species of the genus, is readily differentiated from the three previously known species [*Q. quinqueserialis* (Barker and Laughlin, 1911); *Q. hassalli* (McIntosh and McIntosh, 1934); *Q. wolgaensis*

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[1] Parasitologist, Animal-borne Disease Branch, Arctic Health Research Center, U. S. Public Health Service, F.S.A., Anchorage, Alaska.

Skvortsov, 1935]. The three previously-known species are very similar in most details of their morphology, but differ essentially in number of ventral glands, egg size, and in other, more minor, details. They have two characters in common which set them off clearly from the species described herein: (1) the vitellaria are arranged in longitudinal, mostly extracecal, lateral rows; (2) the uterine convolutions do not pass to any marked extent lateral to the intestinal ceca.

Q. floridensis can be differentiated most readily on the basis of these characters, since in this species the vitellaria consist of two lateral bodies largely posterior to the uterine coils. In this species, moreover, the uterine coils are peculiar in that they extend laterally well beyond the lateral margins of the intestinal ceca and vitellaria and, in fact, in some specimens practically are in contact with the lateral body-margins of the worm. *Q. floridensis* is differentiated further on the basis of ventral gland numbers, and on egg size.

The trematodes of the genus *Quinqueserialis* are typically parasitic in the cecum of microtine rodents. *Q. quinqueserialis* is commonly observed in the muskrat; *Q. hassalli* occurs, usually in relatively small numbers, in the cecum of *Microtus* spp. (*vide* McIntosh and McIntosh, 1934; Harwood, 1939; Rausch and Tiner, 1949; Kuns and Rausch, 1950). *Q. wolgaensis* was also collected from the cecum of a vole, *Arvicola terrestris* (L.) by Skvortsov (1935). Whether *Q. floridensis* n. sp. occurs in other microtine hosts remains to be seen.

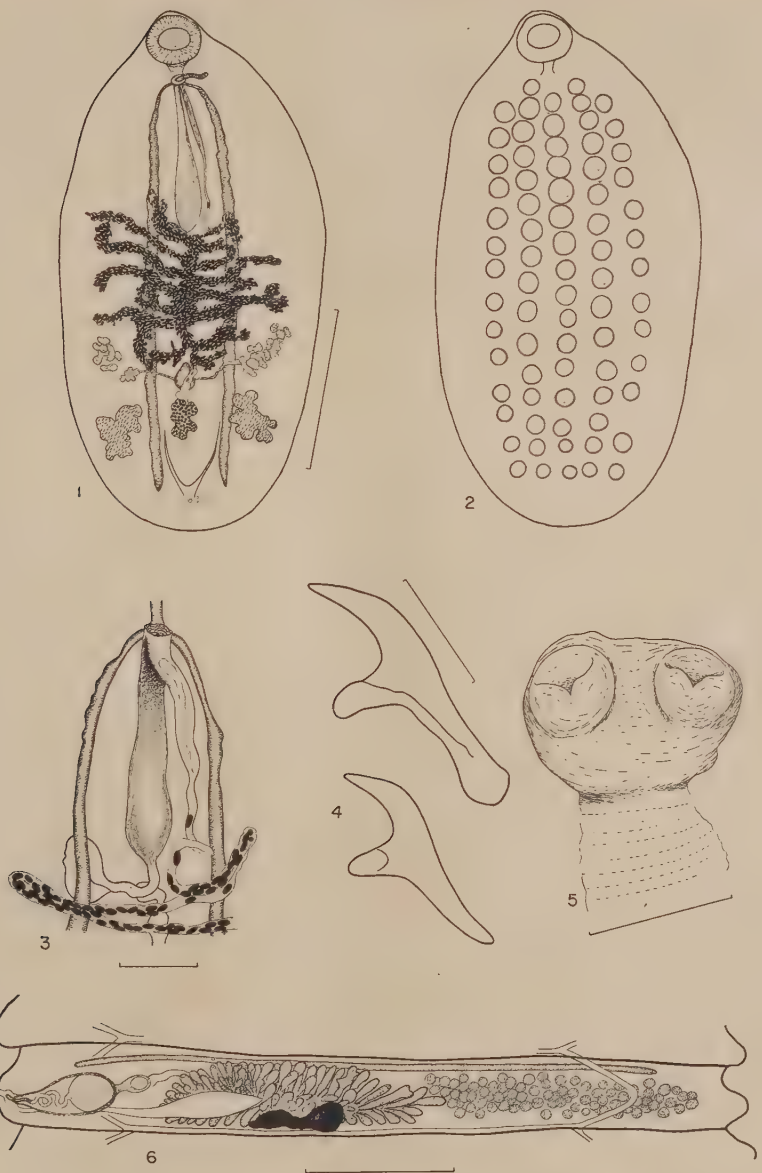
A single cestode of the genus *Cittotaenia* Riehm was removed from the small intestine of one of the rodents. Although gravid and late pre-gravid segments were absent, the strobila measured 65 mm. in length, and 3 mm. in greatest width. In the absence of completely developed eggs, one often cannot assign cestodes of this group with certainty to a given species. However, a study of the morphology of this worm fails to disclose any character which is incompatible with its assignment to the species *C. praecoquis* Stiles, 1895.

C. megasacca was described by Smith (1951) from a pocket gopher (*Thomomys*) from Wyoming. The comparison of the *Neofiber* cestode with specimens of *C. megasacca,* collected by the writer from a pocket gopher, *Thomomys talpoides tenellus* Goldman, from northern Wyoming, has disclosed clear-cut differences. The occurrence of *C. praecoquis* as a parasite in *Neofiber* constitutes a new host record, and considerably increases the known geographical range of this helminth.

Found along with the specimen of *C. praecoquis,* in both the stomach and duodenum of the host, were several specimens of a cestode of the genus *Paranoplocephala* Luehe. Since these cestodes differ significantly from other members of the genus, they are described herewith as a new species. The following description is based mainly on two entire specimens:

Paranoplocephala neofibrinus n. sp.
(Figs. 5–6)

Diagnosis: Strobila length 55 to 56 mm.; greatest width, attained in region of early gravid segments (in last one-fourth of strobila), 5 mm. Strobila, consisting of 180 to 190 segments, strongly attenuated anteriorly. Segments, in mature region, much wider than long (ratio of length to width about 1 : 10) ; length becomes greater in gravid segments, of which the terminal ones have a ratio of about 1 : 3. Scolex, distinctly set off from neck, has diameter of 600 to 750 μ. Suckers round; 250 to 290 μ in diameter. Excretory canals not enlarged. Genital pores unilateral, dextral, situated near middle of segmental margin. Genital ducts dorsal to longitudinal excretory canals. Subspherical testes, about 50 to 60 μ in diameter in mature segments, extend



EXPLANATION OF PLATE

(All figures drawn with aid of projector.)

FIG. 1. *Quinqueserialis floridensis* n. sp.; ventral view, ventral glands omitted. Scale has a value of 500 μ.

FIG. 2. *Q. floridensis* n. sp.; ventral view, showing distribution of glands.

FIG. 3. *Q. floridensis* n. sp.; details of terminal portions of genital ducts. Scale has a value of 100 μ.

FIG. 4. Rostellar hooks of *Taenia ?lyncis* Skinker, 1935. Scale has a value of 100 μ.

FIG. 5. *Paranoplocephala neofibrinus* n. sp.; scolex. Scale has a value of 500 μ.

FIG. 6. *P. neofibrinus* n. sp.; mature segment, ventral view. Scale has a value of 500 μ.

from aporal edge of ovary to well beyond aporal longitudinal excretory canals. Testes, extending through nearly entire length of segment, number about 100; exact count not possible because of their overlapping and close apposition. Ovoid to pyriform cirrus sac prominent, extending across longitudinal excretory canals on poral side; cirrus sac measures from 360 to 380 μ in length by 130 to 136 μ in greatest width in mature segments. Heavily-spined cirrus looped in distal portion of cirrus sac. Large internal seminal vesicle occupies about one-half of cirrus sac. External seminal vesicle well developed. Vagina opens postero-ventral to cirrus sac opening; it may extend somewhat posterior to latter in course of passage medially toward ovary. Seminal receptacle very large in late mature segments, extending aporally beyond middle of ovary. Vitelline gland elongate, variable in shape, situated at base of ovary near middle. Highly-lobed ovary situated almost entirely in poral half of segment, extending in mature segments through entire segmental length. Uterus tubular, extending laterally beyond longitudinal excretory canals; uterine sacculations gradually enlarge, filling entire gravid segment. Eggs measure 38 to 43 μ long by 32 to 43 μ wide (av. 41 by 37 μ). Pyriform apparatus well developed.

Host: *Neofiber alleni nigrescens* Howell.

Habitat: Small intestine.

Locality: Putnam County, Florida.

Type: A slide, No. 47588, bearing an entire specimen has been deposited in the Helminthological Collection of the U. S. National Museum.

Paranoplocephala neofibrinus is differentiated from related species primarily on the basis of egg-size and cirrus sac-size and more detailed characteristics. Referring to the work of Rausch and Schiller (1949a) in their review of North American species of *Paranoplocephala* in *Microtus, P. neofibrinus* is most closely related to *P. variabilis* (Douthitt, 1915). *P. variabilis* has an aspinose cirrus, a smaller cirrus sac, and much smaller eggs (26 to 33 μ long by 20 to 26 μ wide). Grossly the two species are similar, except for the relatively much larger scolex of *P. neofibrinus.* Both also occur in the same habitat—the duodenum of the host.

After the paper by Rausch and Schiller had been published, the description of *P. kirbyi* Voge, 1948, appeared in a journal long delayed beyond scheduled date of publication. In connection with other work, the writer borrowed the type specimen of *P. kirbyi* from the U. S. National Museum, and Dr. Voge very kindly provided additional specimens from the type host, *Microtus californicus.* A careful study of this material revealed that the specimens upon which *P. kirbyi* is based were erroneously assigned to genus, and they actually represent specimens of *Andrya macrocephala* Douthitt, 1915. Such an error might readily enough occur if one misinterpreted the details of uterine formation, since this character, the most important for generic differentiation, often is obscure. That *A. macrocephala* is a very variable species has been shown by Rausch and Schiller (1949b). The morphology of the specimens upon which *"P. kirbyi"* is based will be discussed further, more appropriately, in another publication (Rausch, 1952).

Among Eurasian species of *Paranoplocephala, P. omphalodes* (Hermann, 1783) is the only species, on the basis of published descriptions, which *P. neofibrinus* might resemble. However, through the study of comparative material, and because of discrepancies in the published descriptions, *P. neofibrinus* is considered clearly distinct from *P. omphalodes.* Although reported by Harkema (1946) from the cotton rat (*Sigmodon*) in North Carolina, *P. omphalodes* is not known to occur in the United States. The original identification of this cestode was erroneous (cf. Harkema and Kartman, 1948; p. 185: *Andrya microti* Hanson). This earlier published record was unfortunately not clarified by Harkema and Kartman in their later publication on the parasites of the cotton rat. *P. omphalodes* was recorded from arctic Alaska by Rausch (1951); the occurrence of this species in North America will be discussed in another publication (Rausch, 1952).

One of the round-tailed muskrats examined in connection with this study showed a heavy liver infection by larval cestodes belonging to the genus *Taenia* L. Sixteen cysticerci were observed, localized on both the dorsal and ventral surfaces of the organ. The cysts measured, on the hepatic surface, 3 to 4 mm. in diameter. The cysticerci possessed from 40 to 44 rostellar hooks (Fig. 4). The large hooks measured from 220 to 244 μ in length, and the small hooks measured from 172 to 187 μ in length.

In the absence of adult cestodes for detailed morphological study, specific identification of these cysticerci can hardly be made. A review of available information on hook details of cestodes of the genus *Taenia* indicates that the hooks of these cysticerci closely resemble those of *T. lyncis* Skinker, 1935. Harkema and Kartman (1948) tentatively identified as *T. lyncis* a cysticercus from the liver of a cotton rat. Skinker (1935) recorded deer and a white-footed mouse (*Peromyscus*) as possible intermediate hosts of this cestode, but considered controlled feeding experiments necessary to establish with certainty the identity of such larvae. Joyeux (1945) also figured the hooks of *Taenia* sp. from *Felis macrura,* of Brazil. These hooks also closely resemble those of *T. lyncis.* The study of adult cestodes from Florida carnivores will be necessary to establish whether *C. lyncis* is present in that region.

SUMMARY

Two specimens of round-tailed muskrat, *Neofiber alleni nigrescens* Howell, were examined for helminth parasites. Four species, a trematode and three cestodes, were collected. Two of these, *Quinqueserialis floridensis* and *Paranoplocephala neofibrinus,* are described as new. *Cittotaenia praecoquis* Stiles, 1895, is recorded from this host, and larval cestodes are tentatively identified as *Taenia lyncis* Skinker, 1935.

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A NEW XIPHIDIOCERCARIA, *C. GOODMANI,* FROM *LYMNAEA PALUSTRIS*[1]

HAIG H. NAJARIAN

Herein is described a new Xiphidiocercaria, *Cercaria goodmani,*[2] n. sp. Characteristics of *C. goodmani* place it in Sewell's (1922) modified Polyadena group and more specifically in Brooks' Conniae division of Polyadena.[3]

MATERIALS AND METHODS

During the months of April through October, 1951, 788 specimens of *Lymnaea palustris* were collected from a series of temporary ponds along Zebe Road, six miles west of Ann Arbor, Michigan. These specimens were isolated in small bottles of aërated tap water and observations made on the shedding of cercariae. No attempt was made to crush the non-shedding snails in order to determine the total percentage infection. The cercariae were studied in the living state. The usual methods of intra-vitam staining with Nile-blue sulfate and neutral red were used. The urine method of West (1935) proved of no great aid in studying the excretory system. The temporary mount of Goodman (1951), using a mixture of glycerine, formaldehyde and potassium hydroxide proved very useful in measurements. Refrigerating overnight and study the next morning aided greatly in the examination of the excretory system. The change of temperature apparently enhances the activity of the flame cells. An even more useful method in determining the flame cell pattern and one which particularly aided in the study of the penetration glands was the crushing of infected snails under a coverglass water mount. The snail's body fluid seemed to have some dynamic action upon flame cell activity and rendered the penetration glands more distinguishable. This study suggests it as a successful medium for studying living cercariae. Dilute Semichone's aceto-carmine was used for studying the genital anlage.

SNAIL INFECTIONS

Of the 788 specimens of *L. palustris* examined, 92 or 12.6% showed infection with *C. goodmani.* Cercariae of *Schistosomatium douthitti* Cort 1914, an undetermined echinostome cercaria, and a furcocercous cercaria believed to be the larval form of *Clinostomum attenuatum* Cort 1913 were also found. No mixed infections were observed. Other snails from the same ponds, viz. *Lymnaea caperata, Physa gyrina, Aplexa hypnorum,* and *Gyralus parvus* were not infected with *C. goodmani.* Collection data showed the following infection:

Month	*Number collected*	*Per cent infected*
April	32	21.9
May	155	16.6
June	360	10.5

Received for publication, November 30, 1951.

[1] Contribution from the Department of Zoology, University of Michigan.

[2] Named in honor of Dr. John D. Goodman, University of Redlands, Redlands, California.

[3] Sincere acknowledgment is extended to Dr. Arthur E. Woodhead, under whom this investigation was made, and to Dr. John D. Goodman for his kind assistance during the study.

Month	*Number collected*	*Per cent infected*
July	96	9.0
August	51	15.0
September	10	0.0
October	84	16.6

Cercarial shedding probably takes place both prior to April and subsequent to October but to what extent was not determined by this study.

Although not all the snails were measured, the infected snails prior to the October collection had a shell length (bottom of aperture to tip of spire) of 19–35 mm. Many of the infected snails collected in October were as small as 15 mm. These small specimens may have come from eggs laid that year and the large snails of the spring collections may have carried infections over from the previous fall.

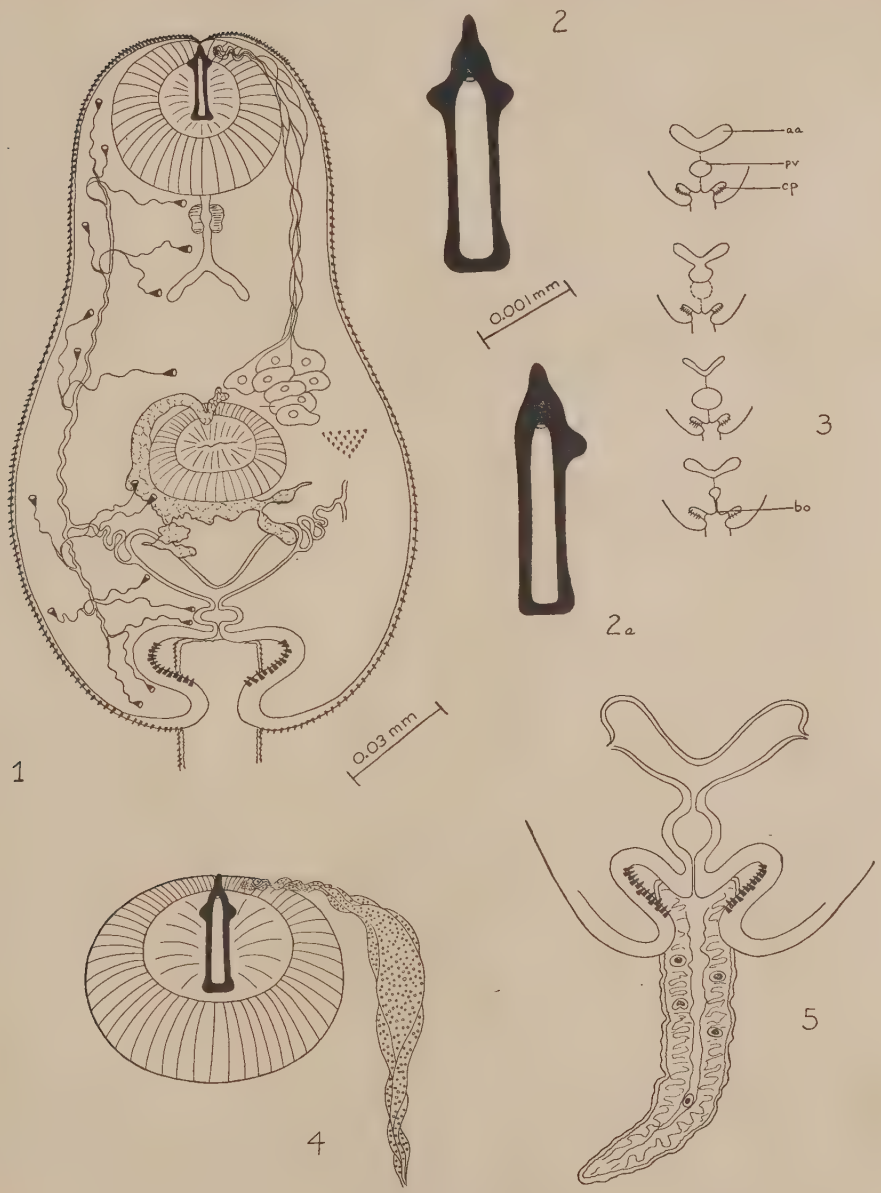
Cercaria goodmani, n. sp.

This species of cercaria, once shed from the snail, remains alive for as long as 48 hours. It is negatively geotropic. It exhibits two types of motion in water, a creeping action on a flat surface and an upward swimming motion, the latter being the most frequent type of motion. Just before the cercaria begins to swim, the body is flexed so that the posterior portion forms one arm of a U-shaped figure, the tail being fully extended. During the vibratory movements of both body and tail there is a stationary node at the middle of the body and one at the anterior third of the tail.

Diagnosis: (Measurements on living cercariae, in millimeters.) Xiphidiocercaria of the Conniae division of the Polyadena group; body oval or somewhat pear-shaped, length—0.180, width—0.082, flexible, capable of great contractions; unpigmented; eyespots lacking; stylet javelin-shaped, length—0.027, width—0.006, heavy shoulders one-fourth distance from anterior end, re-enforced sides and base, with lateral expansions on the latter, basal bulb lacking, shaft in center with small, oval aperture at anterior end; numerous cystogenous glands especially in region of pharynx; numerous oil-like globules; eight large penetration glands on each side both lateral and anterior to acetabulum, ducts of penetration glands with separate openings on lateral surface near tip of stylet; prepharynx, pharynx, and esophagus of equal lengths, viz. 0.014, pharynx width 0.010; ceca extremely short and terminating far anterior to acetabulum, characteristic indentations on ceca often apparent; acetabulum somewhat smaller than oral sucker and located slightly posterior to middle of body, oral sucker diameter—0.058, acetabulum diameter—0.036; conspicuous caudal pockets with 13–20 spines on lateral surface of each pocket; cuticula spinose, spines directed posteriorly and arranged in transverse rows forming a diamond-shaped pattern, spines cover entire body and tail; genital anlage prominent when stained in Semichone's, cirrus sac lateral and dorsal to acetabulum; testes side by side at level of bladder arms; ovary anterior to testes, posterior and lateral to acetabulum; tail not as long as body except in extremely extended condition, tail length—0.105, tail width—0.041, attached subterminally to ventral body wall, tail with many large nucleated cells, prominent medial canal-like structure; excretory bladder bicornate, opening at base of tail, main excretory ducts subterminal and highly convoluted just after receiving anterior and posterior branches; flame cell pattern $2[(3+3+3)+(3+3+3)]=36$ flame cells. The bladder consists of two anterior arms connected by a slender canal to an oval posterior vesicle which opens into the caudal pocket region by another slender canal (Fig. 3). The operations of the bladder are such that there is an alternate filling and emptying of the anterior arms and the posterior vesicle. Develops in elongate sporocysts 0.302–0.340 mm. × 0.041–0.047 mm. often with swellings at one end; massed within snail's digestive gland; sporocyst wall with numerous orange granules.

Relationships:

Lühe (1909) erected the Armatae group to include those Xiphidiocercariae which did not possess a fin-fold and whose body length was greater than 250 μ. Cort (1915), on the basis of size and possible life history affinities, created the Polyadena group. Sewell (1922) modified Cort's Polyadena group and placed it as a subgroup of Armatae. Brooks (1943) suggested the erection of a Conniae division of Polyadena to include those cercariae having javelin stylets, lacking basal bulbs and fin-folds.



EXPLANATION OF PLATE

FIG. 1. *Cercaria goodmani,* n. sp., ventral view; excretory pattern on left; penetration glands on right.

FIG. 2. Stylet, dorsal view.

FIG. 2a. Stylet, side view.

FIG. 3. Sketches showing successive stages in operation of excretory bladder; from living material. aa—anterior arm, pv—posterior vesicle, cp—caudal pocket, bo—bladder opening.

FIG. 4. Sketch showing swellings of penetration gland ducts before opening in region of anterior sucker.

FIG. 5. Sketch showing subterminal attachment of tail; prominent spines in conspicuous caudal pockets; prominent contractile elements in tail with several large nucleated cells.

Although the characteristics of *C. goodmani* place it in Brooks' Conniae division, it shows some morphological relationships with the following seven cercariae: *Cercaria indicae XVII* Sewell 1922, *Cercaria conniae* Brooks 1943, *Cercaria nolfi* Brooks 1943, *Cercaria concavacorpa* Sizemore 1936, *Cercaria isocotylea* Cort 1914, *Cercaria acanthocoela* Miller 1935, *Cercaria micropharynx* Faust 1917. Of these, it most closely resembles *Cercaria micropharynx* but differs from it in the following features. The sporocysts of *C. micropharynx* are oval, measuring 0.24 mm. × 0.18 mm., those of *C. goodmani* are elongate, measuring 0.340 × 0.047 mm. *C. micropharynx,* if pressed out of the sporocyst, swims for only a brief period and then drops off its tail and encysts. *C. goodmani* is capable of swimming for as long as 48 hours and was never seen encysting in an aqueous medium. Very conspicuous caudal pockets are present, which upon first glance might be mistaken for the lower portion of the excretory bladder, while caudal pockets of *C. micropharynx* are less conspicuous. Faust described the pharynx of his species as very minute but gave no measurements. The pharynx of *C. goodmani* is 0.014 mm. long and is not considered minute. Three of the penetration glands of *C. micropharynx* are located posterior to the acetabulum while in *C. goodmani* all of the glands are located lateral or anterior to the acetabulum. Faust's figure shows only seven glands on the right side but gives no explanation concerning this. The stylet of *C. micropharynx* is less re-enforced at the base.

The two main excretory ducts of *C. goodmani* enter subterminally into the Y-shaped bladder. The main branches of *C. micropharynx* enter the terminal portion of the bladder arms and a tail excretory tube "with inconspicuous lateral tributaries" is described. No similar structures were observed in *C. goodmani,* its bladder opening in the medial subterminal region of the caudal pockets. However, a "canal-like" structure was observed running almost the entire length of the tail, but its exact nature is uncertain. Cort (1919), in his description of the excretory system of *C. polyadena,* stated that no part of the excretory system extends into the tail, and further stated that in his studies of the excretory system of stylet cercariae no trace of the excretory tubes were ever seen in the tail. Hussey (1941) in her study of the excretory system of digenetic trematodes, stated that "since in development, the excretory tubes in stylet cercariae never enter the tail, it is possible that muscular elements have been mistaken for an excretory tube and figured as such."

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CERCARIA MYAE SP. NOV., A FORK-TAILED LARVA FROM THE MARINE BIVALVE, *MYA ARENARIA*

Joseph R. Uzmann
U. S. Fish and Wildlife Service, Milford, Conn.

Cercaria myae has been found in three of 910 specimens of the soft clam, *Mya arenaria,* collected in the vicinity of Newburyport, Mass. Two infections were found upon dissection and examination of 143 preserved specimens which had been collected in July, 1949. The remainder of the material consisted of many smaller collections of living specimens. These, too, were examined by dissection since the material was being utilized for several independent studies. The third infection was found in a May, 1951 collection and the description to follow is based mainly upon that source.

The cercariae are produced in motile, unpigmented sporocysts which occupy the interfollicular spaces of the gonad and the interlobular lymph spaces of the digestive gland; lesser numbers of sporocysts occur throughout the hemocoel. The gonad is the principal focus and severe atrophy of that diffuse organ is evident in sectioned material. There is histological evidence of partial to complete sterilization. In one of the earlier examples, the infection was so extensive that it is considered doubtful that similarly infected animals could survive abnormal conditions.

Cercariae were observed to emerge spontaneously from sporocysts which had been teased from the host tissues and placed in watch glasses containing sea water at room temperature. The sporocysts are typically clavate with thin walls and a birth pore in the apical end; they vary in length from 0.21 to 0.60 mm. The cercariae emerge either head or tail first after which they remain quiescent for several seconds. In swimming, the posterior portion of the body is bent ventrally in apposition to the anterior portion whereupon a vigorous lashing of the tail causes an inversion of the animal and a rising, random movement. The cercariae did not encyst and became moribund after about twelve hours. Owing to the limited amount of living material, exposure trials were not attempted.

The cercariae (Fig. 1) are small with elongate-ovoid bodies and a forked tail. The length of the body varies from 0.12 to 0.25 mm. in contracted and extended condition. The tail stem is short, about one third the length of the body, and slightly shorter than the furcae. The body is covered with minute spines less than 0.001 mm. long, and delicate setae occur on the posterior surfaces of the furcae. The oral sucker is relatively large, spherical to oval, and measures 0.039 to 0.052 mm. in diameter. It is followed almost immediately by a pharynx approximately half as large in diameter (0.020–0.025 mm.). A short esophagus quickly flares into large digestive ceca, the walls of which are composed of large polygonal cells. A small, deeply staining, spiral coil of supporting material maintains a constant lumen between the pharynx and esophagus; this structure is best seen in stained sections. The acetabulum is approximately as large as the oral sucker and varies from 0.040

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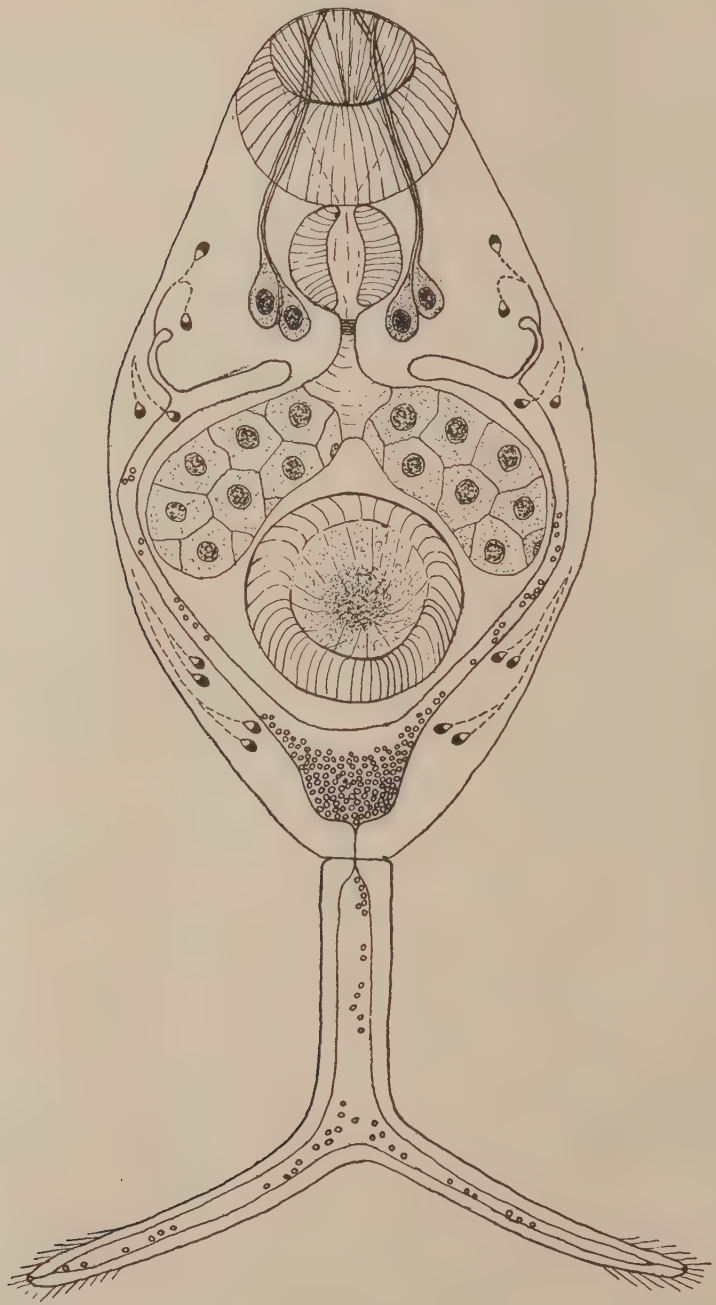

FIG. 1. *Cercaria myae*, ventral view.

to 0.046 mm. in diameter. Two pairs of penetration glands, with well defined ducts, are arranged in bilateral fashion near the posterior level of the pharynx. The ducts on either side are closely applied as they pass forward to the dorsal margin of the oral sucker, but diverge abruptly to open in four separate pores. The excretory vesicle is U-shaped with long arms that extend forward and bend mediad around the digestive ceca, terminating blindly near the penetration glands. Primary tubules arise near the ends of the arms. Sixteen flame cells have been located, but their connections could not be traced with certainty. The excretory vesicle opens into an enlarged caudal duct which traverses the tail stem, divides, and continues axially to the tips of the furcae.

Cercaria myae belongs in the group of Furcocercous Cercariae. Attempts to relate it to a distinct taxonomic group must await a knowledge of its post-larval form. It is interesting to note, however, the close morphological agreement between this form and another unclassified larva, *Cercaria discursata* Sinitsin, 1911, which develops in sporocysts in the gonad and liver of the marine bivalve *Abra* (= Syndosmya) *alba* of the Black Sea. In contrast to *C. discursata,* which does not swim and sheds its well-developed forked tail while still within the sporocyst, *C. myae* manifests a pronounced natatory ability. Apart from this difference and certain size discrepancies, comparison of the two suggests intrageneric relationship.

Stafford (1912) may be credited with the original observation of *C. myae* on the basis of a brief description which appears in a discussion of human illness and fatality, ascribed to shellfish poisoning. ". . . in a single case out of a very large number the whole abdomen of a clam (*M. arenaria*) was distended, soft, translucent, and pale yellowish-green in colour, while a puncture occasioned a copious flow of an aqueous fluid containing hundreds of *sporocysts,* most of which inclosed about 20 full-formed split-tailed *cercariae.* The body of one of these measured .138 × .082 mm. . . ." He hesitatingly suggested, without evidence, that this form could have been the basic cause of the shell-fish poisonings. It should be noted here that the fundamental cause of shellfish poisoning is believed to be associated with several species of dinoflagellates which appear in the planktonic food of molluscs. Needler (1949) presented data and reasons for regarding *Gonyaulax tamarensis* as the principal cause of shellfish toxicity in the Bay of Fundy area. Stafford's description of the cercaria, however, is suggestively diagnostic and in all probability refers to the species described here.

Allison (1943) experimentally traced the life cycle of *Leucochloridiomorpha constantiae* (Mueller, 1935), a brachylaemid trematode having natatory furcocercous cercariae which develop in an aquatic snail. Since all other known brachylaemid cercariae develop in terrestrial snails and have very abbreviated tails, he postulated that *L. constantiae* represents a surviving ancestor of the BRACHYLAEMIDAE. He suggested that *C. discursata* may possibly belong to the BRACHYLAEMIDAE and, in that case, would represent a form intermediate between the tailed and tailless cercariae of the family. If Allison is correct, *C. myae* could be tentatively referred to the same family. Further study of both species is highly desirable and may provide important information concerning the phylogeny of the BRACHYLAEMIDAE.

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THE LIFE CYCLE OF *CONSPICUUM ICTERIDORUM* DENTON AND BYRD, 1951, (TREMATODA: DICROCOELIIDAE)

JOHN A. PATTEN
University College, New York University

INTRODUCTION

The family DICROCOELIIDAE Odhner, 1910, was erected to include those elongate, transparent to translucent distomate trematodes parasitic in the gall bladders, bile ducts, and sometimes the pancreatic ducts, of amphibians, reptiles, birds, and mammals. Two subfamilies were recognized:

1) BRACHYCOELIINAE Looss, 1899, which contains forms parasitic in amphibians and reptiles.

2) DICROCOELIINAE Looss, 1899, which contains forms parasitic in birds and mammals.

No life cycle data are available for members of the subfamily BRACHYCOELIINAE.

Knowledge of the life history of trematodes in the subfamily DICROCOELIINAE has accrued with painful slowness and is so inadequate as to be of little value for taxonomic purposes. This dearth of knowledge pertaining to developmental stages has compelled workers to rely on other less desirable and less dependable bases for assignment, not the least of which have been the use of unstable and minor structural features, resulting in a tendency to disregard the effect of the host-parasite relationship on gross morphology of the parasite and the far-reaching impact this may have upon taxonomic schemes. Many workers, including Dollfus (1922), on *Dicrocoelium dendriticum;* Ware (1923), on *Platynosomum fastosum* and *Eurytrema pancreaticum;* Beaver (1937), on *Echinostoma revolutum;* Rankin (1938), on the subfamily BRACHYCOELIINAE; and Willey (1941), on *Zygocotyle lunata,* have pointed out size or other morphological variations within a given species. Furthermore, the absence of vital information on life histories has led to considerable duplication of effort with a variety of interpretations, resulting synonymy, and, it might be said, confusion in general. This condition is well exemplified in the genus *Eurytrema* as pointed out by Stunkard (1947). It is believed, therefore, that the true relationship of forms can be established only after much more experimental data have been presented and the large group of dicrocoeliid parasites can be reexamined in the light of such knowledge.

Research pertaining to the subfamily DICROROELIINAE has been principally concerned with revision of the taxonomy of the group and the description and assignment of new species. Since the present paper is limited to the study of a single species of the genus *Conspicuum* Bhalerao, 1936, no attempt has been made to present a comprehensive review of other genera in the Dicrocoeliinae. However, a brief

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review of the literature pertinent to the derivation of the genus *Conspicuum* is included. Bhalerao (1936), in a study of trematodes of India, reconsidered the genus *Eurytrema* and recognized in that group five subgenera: *Pancreaticum, Concinnum, Conspicuum, Skrjabinus,* and *Lubens.* Strom (1940), reexamined material from Europe and Asia and made several changes relative to the genus *Eurytrema.* He removed all avian parasites from the genus and elevated Bhalerao's subgenera to full generic rank. Travassos (1944), apparently unaware of Strom's paper, also raised the subgenera *Concinnum* and *Conspicuum* of Bhalerao to generic rank but retained *Lubens* and *Skrjabinus* as subgenera. Since the revision proposed by Strom antedates that of Travassos, the changes as set forth by Strom have priority. Bhalerao (1936) included in the subgenus *Conspicuum* those forms with the genital pore anterior to the intestinal fork, uterus confined to the posterior half of the body, vitellaria occupying nearly half of the body length, and ventral sucker larger than the oral sucker. He assigned two species, *Eurytrema conspicuum* de Faria, 1912 and *Eurytrema pulchrum* Travassos, 1919 to the subgenus. Travassos (1944) included also *Conspicuum acuminatum* (Nicoll, 1915) and Denton and Byrd (1951) added two new species, *Conspicuum macrorchis* and *Conspicuum icteridorum.* The species with which this paper is concerned is recognized as *Conspicuum icteridorum* Denton and Byrd, 1951.

In the family Dicrocoeliidae knowledge of the life history is known only in the subfamily Dicrocoeliinae where complete details are available for a single species, *Dicrocoelium dendriticum.* Incomplete data exist for three others, *Concinnum procyonis* (Denton, 1942) Travassos, 1944; *Brachylecithum americanum* Denton, 1945; and *Platynosomum fastosum* Kossak, 1910. The first life cycle to be completed for a member of the subfamily Dicrocoeliinae was that of *D. dendriticum* and this was accomplished only after many years of research by a number of investigators. *Cercaria vitrina* von Linstow, 1887, was incompletely described and was not identified with the life cycle of *D. dendriticum* until Vogel (1929) restudied the cercaria and pointed out that structural details identified it as the larval stage of *D. dendriticum.* His preliminary investigations indicated the necessity of a second intermediate host. Cameron (1931) performed the first step in the experimental approach to the life cycle. He fed eggs of *D. dendriticum* to a terrestrial snail, *Helicella itala,* and obtained cercariae identified as *Cercaria vitrina.* Henkel (1931) made additional studies of the intramolluscan development. Mattes (1936) repeated the experiments of Cameron and studied the intramolluscan development of *D. dendriticum* using, instead of *H. itala,* three other terrestrial mollusks, *Helicella erecetonium, Helicella candidula,* and *Zebrina detrita.* Neuhaus (1936, 1938) demonstrated that no second intermediate host was involved in the transfer of the cercariae. Instead, they collected in large groups in the mantle cavity of the snail and from the contents of their larger glands formed common spherical cysts which accumulated in grape-like clusters near the respiratory pore and became covered with slime from the snail before they were deposited. The slime hardened and formed additional protection around the cercariae, and the definitive host ingested the cysts while feeding. The cercariae were liberated in the intestine, penetrated its walls and traveled by way of the portal system to the liver, thus completing the cycle.

Denton (1941) reported preliminary studies on the life cycle of *Lyperosomum*

sp. Later (1945), he identified the species as *Brachylecithum americanum* and gave additional details of the life history. The parasite occurs in the biliary ducts of birds belonging to the families CORVIDAE and ICTERIDAE. The molluscan hosts were established as *Polygyra texasiana* and *Practicollela berlandieriana.* Eggs were fed to these snails and mother sporocysts developed and gave rise to a limited number of daughter sporocysts. Cercariae were matured successively within the daughter sporocysts, from which they escaped by way of a birth canal. They collected within the mantle cavity of the snail and were expelled through the respiratory pore in masses of 150–300. Intramolluscan development was reported to be similar to that described for *D. dendriticum* and the cercariae were said to resemble those of *D. dendriticum* in basic morphology, including the possession of a well-developed tail.

Denton (1944) attempted to work the life cycle of *Concinnum procyonis* (= *Eurytrema procyonis*), a parasite of the interlobular pancreatic ducts of the raccoon, *Procyon lotor.* Asexual reproduction of this species occurred in *Mesodon thyroidus.* Mother sporocysts gave rise to daughter sporocysts over a long period of time and within each of the latter, 20–40 cercariae were sealed. The daughter sporocysts collected in the mantle cavity of the mollusk and were discharged by way of the respiratory pore. The daughter sporocysts as described differed from those of *D. dendriticum* in morphology and in the manner and number of cercariae produced. The cercariae of the two species were reported as similar in their fundamental morphology, although it may be of importance to note that the cercariae of *C. procyonis* possess short, rudimentary tails whereas those of *D. dendriticum* are long and well developed. The mode of infection of the definitive host was not established for either *B. americanum* or *C. procyonis* but it was suggested by Denton (1945) that, since attempts at direct infection of final hosts were unsuccessful, insects of the family CHRYSOMELIDAE might serve as second intermediate hosts for *B. americanum.*

Maldonado (1945, 1946) reported the second (complete except for certain details) life history from the subfamily DICROCOELIINAE. He confirmed the observation of van Volkenberg (1937), that the snail, *Subulina octona,* is the molluscan host for *Platynosomum fastosum* Kossak, 1910, and reported on further stages in the life cycle of this trematode commonly found in the gall bladders of cats in Puerto Rico. The mother sporocysts, as reported, developed within the gonads of the snail and gave rise to daughter sporocysts over a long period of time. Within each daughter sporocyst there developed simultaneously an average of 17.5 cercariae. The sporocysts, with their imprisoned cercariae, were shed as in *C. procyonis* by way of the respiratory pore of the snail. The cercariae were brevicaudate and as described by Maldonado (1945, 1946) conform in all major details to the description of the cercariae of *C. procyonis* as given by Denton (1944). Unencysted, developing, "metacercariae" were found in the common bile duct of the lizard, *Anolis cristatellus,* but the manner in which the lizard acquired the infection was not determined, and the cycle was completed when infected lizards were fed to cats.

The present life history study presents new information which should make possible the early solution of pending life history problems in the family Dicrocoeliidae. *Conspicuum icteridorum* is a parasite of the gall bladder of birds of the family ICTERIDAE. It is common in the purple grackle, *Quiscalus quiscula quiscula* Linneaus. The first intermediate host is here established as *Zonitoides arboreus*

(Say). Denton and Byrd (1951) reported that *Deroceras laeve* (Müller) may serve as a molluscan host of this species. The second intermediate hosts are experimentally established as land isopods *Oniscus asellus* Linnaeus and *Armadillidium quadrifrons* Stoller. Possibly other isopods may serve as well, and it is not improbable that a variety of arthropods may be involved in the great complex of developmental histories among the dicrocoeliid parasites.

MATERIALS AND METHODS

Because purple grackles are fairly common in the Bronx Zoological Park, can be obtained without great difficulty, and present a high incidence of infection with *C. icteridorum,* it was decided to attempt to work the life cycle of this parasite.

Since the asexual stages of digenetic trematodes occur in mollusks, a survey of the species in the area was undertaken. The grackle is an omnivorous feeder and accordingly both terrestrial and aquatic mollusks were checked for possible infections. Of the aquatic species, *Helisoma anceps* were found to be harboring *Cercaria poconensis* Willey, 1930, a larval stage of *Zygocotyle lunata; Physella heterostropha* were infected with the cercariae of *Echinostoma revolutum* (Froelich, 1802), and with *Cercaria burti* Miller, 1923, a larval stage of the strigeid, *Apatemon gracilis.* The terrestrial mollusks taken were *Anguispira alternata* (Say), *Zonitoides arboreus,* and a species of small, as yet unidentified, slug. None were naturally infected.

Grackles were maintained on a diet of cracked corn, grated carrots, chopped apples, and hard-boiled eggs. Infected birds were sacrificed and eggs were obtained either from the gall bladder, where they were shed naturally in great numbers, or they were teased from the uteri of mature worms. In the first series of experiments, the terrestrial mollusks mentioned above were used in groups of five, and approximately fifty eggs were mixed with the food (rolled oats and finely ground corn meal) of each group. All experimental groups were kept in small containers which were first lined, top and bottom, with paper toweling and filled to a depth of one-half inch with humus and decaying wood. The contents of the containers were kept moist and the food was changed frequently. Infections developed only in *Z. arboreus* and mature sporocysts were shed.

All dissections of snails and all in vivo studies of larval stages were made in Ringer's solution under a dissecting microscope with a magnification of 43 diameters. Detailed studies of living material were made with a compound binocular microscope, and with a standard microscope using phase equipment. Stained and unstained materials were used. Intra-vitam dyes utilized were neutral red, Nile blue sulfate and brilliant cresyl blue. Duplicate material was fixed in hot formalin, Bouin's solution, or Gilson's fixative and was stained with Semichon's carmine. A camera lucida attachment was used whenever feasible for making outline drawings and a *Visicam* (trade name) photomicrographic camera was used extensively to record form and mobility of living stages.

While studies of the intramolluscan stages were in progress, the question of a possible second intermediate host was attacked. Grackles which had been in captivity for several weeks were forcibly fed, by means of a pipette, sporocysts from the experimental infections and were autopsied at various intervals up to one month thereafter. Infected snails were fed to birds. Other snails were given food

containing sporocysts in efforts to determine whether the same snail could also serve as a second intermediate host, and these snails were fed to the grackles. All results were negative. Since Denton (1941, 1945) suggested insects as possible intermediate hosts for *Lyperosomum* sp. (= *B. americanum*), insects were exposed to cercariae of *C. icteridorum.* These included laboratory-raised grain beetles, *Tenebrio molitor* (larvae and adults), spotted lady-bird beetles, Japanese beetles, and grasshoppers. No metacercariae were found in the insects. Maldonado (1945, 1946) reported the lizard, *Anolis cristatellus,* as the second intermediate host of *Platynosomum fastosum,* a dicrocoeliid species infecting cats in Puerto Rico. A search for lizards was made in the Park but none were found.

Because random attempts at finding a second intermediate host had failed, a search was begun for a likely host among animal forms living in natural association with *Z. arboreus.* This snail is largely restricted to a life in, on, or about decaying logs, stumps, and trees and since it does not migrate widely and the sporocysts are attached after deposition, it seemed logical that the second intermediate host must be found in the immediate vicinity of the shedding snail. Myriapods, bark beetles and their larvae, mites, and isopods were the major animal forms investigated. These were exposed to infection with *C. icteridorum* by placing sporocysts on their food and by restricting the infected snail and the possible host to the same container. Examination after seven days showed that one of three isopods of the species *Oniscus asellus* harbored six metacercariae, while another contained five. Later experiments, involving laboratory raised isopods of the species *Oniscus asellus* and *Armadillidium quadrifrons,* established an infection rate of approximately 70% in each of the two species and the metacercariae were positively identified with the cercariae. Thus it was first definitely determined that a dicrocoeliid trematode of birds requires an arthropod second intermediate host to complete its life cycle.

The cycle was concluded by feeding metacercariae to grackles and recovering worms from the gall bladders. These decisive infection experiments were carried on with young birds which were maintained in captivity for periods up to three months. Metacercariae seventy-days old were teased from their cysts and studied in detail. Other metacercariae from the same isopods were fed to birds which had been in captivity a minimum of 10 days. Sixteen hours after the exposure the birds were sacrificed and young worms were recovered from the gall bladders. These worms were in every respect, including measurements, identical with the metacercariae. The rapidity with which the metacercariae arrived in the gall bladder makes it evident that there was no period of growth or residence in the sinuses of the liver or in the biliary ducts. The fact that the stylet was absent in the 70 day old metacercariae indicates that the metacercariae did not penetrate the wall of the intestine and the blood vessels of the bird to follow the circulatory route to the gall bladder. Instead, it is indicated that they followed the most direct route, namely the bile duct, to the gall bladder. Other birds were given several exposures at weekly intervals. Worms were recovered in correspondingly successive stages of development. One bird was hand raised from a nestling so that the probability of its having become naturally infected was greatly reduced. Twelve weeks after it was experimentally exposed a few fully embryonated eggs of the parasite were being passed. Thus it seems that the minimum of 12 weeks is required for the worms to reach sexual ma-

turity. However, normal adult body size may be reached several weeks earlier. This observation is further borne out by data obtained from naturally infected juvenile birds. Eggs of the parasite were first recovered from these birds only in late summer or early fall, at least three months after the nesting season. Efforts to infect chicks, ducklings, canaries, and pigeons were unsuccessful.

EXPERIMENTAL PROCEDURE AND OBSERVATIONS

Since, of the mollusks exposed, only *Z. arboreus* developed an infection, efforts to infect other mollusks were discontinued and observations on development of the parasite were confined to a series of experiments with laboratory raised *Z. arboreus*. Snails were exposed in groups, each of which consisted of 5–10 individuals and infections developed in all groups. At the time of this writing the path of the miracidium within the snail and the early development of the parasite have not been determined. However, the digestive gland of the mollusk appears to be the primary seat of localization, the mother sporocyst developing there is a whitish, granular mass of irregular outline. By the 70th day of development the mother sporocyst has greatly expanded and occupies most of the dorsal surface of the body of the host. Removal of the shell at this age released several hundred immature secondary sporocysts and many more could be seen in almost every part of the body. At approximately 95 days after exposure the *Z. arboreus* began shedding mature secondary sporocysts. These were extruded by way of the respiratory pore. The daughter sporocysts were deposited on the bottoms of the terraria singly (rarely otherwise) and almost invariably with the long axis perpendicular to the substratum to which they were attached. In freshly released sporocysts the cercariae could be clearly observed in a cluster at the attached end of the sporocyst where they slid back and forth producing a flickering effect when viewed under low magnification and causing a slight but sometimes detectable movement of the entire structure. Independent movement of mature daughter sporocysts was not observed. Whether the reflection of light from the moving cercariae or the movement of the sporocyst, or both, were instrumental in attracting the second intermediate host was not determined but it seems plausible that they were. The walls of freshly released sporocysts were elastic and somewhat resistant to pressure, but in older specimens slight pricking or mechanical force caused the outer layer to rupture into a fluid mass about the inner part of the sporocyst which consisted of a sealed brood sac containing the cercariae. Since there is no opening for escape from the brood sac, or endosac, it is almost certain that this structure with its cercariae is taken intact by the second intermediate host and the liquid medium produced by the breakdown of the outer wall of the sporocyst serves as a lubricant in the swallowing process. The sporocysts maintained their turgidity for about two days under moist conditions and the cercariae remained active until well into the third day. However, the transparency of freshly deposited sporocysts was gradually replaced, with age, by opacity until a milky color was reached and concomitant with this there was a shriveling of the outer layer about the inner brood sac.

There was nothing to indicate that in the snail light acted as a stimulus to the migration of the secondary sporocysts and to the shedding process. Neither did there seem to be periodicity in the discharge of sporocysts. Snails kept in darkness and in light deposited about equal numbers of sporocysts for a given period of time.

It is thought that the daughter sporocysts actively migrated to the respiratory chambers of the snails but it appears that the final step, the actual discharge from the mollusk, was accomplished by activity on the part of the host alone. Whereas there was no periodicity in the shedding of sporocysts, there was a direct relationship between moisture content of the terraria and shedding, and between the food and activity of the mollusks and the number of sporocysts released. In the event of low relative humidity the snails became dormant and few or no sporocysts were deposited but with an increase in humidity to near the saturation point, and with the presentation of a fresh supply of food, the mollusks became more active and the number of sporocysts released was increased. Snails with recently matured infections discharged more sporocysts than those with long established infections. Mollusks which had been shedding for three months deposited an average of five sporocysts per twelve hour interval. Snails continued to shed throughout their lifetime.

It was not determined how the cercariae are liberated within the isopod, but since there appears to be little probability that they can obtain sufficient traction to attack the endosac wall and liberate themselves, it seems more likely that they are freed by digestive action of the isopod. The possibility of the endosac being ruptured by chewing action of the isopod should not be ruled out. After the cercariae have penetrated the wall of the digestive tract and have entered the haemocoel they do not encyst at once but migrate through the tissues for at least several hours and perhaps for a day or two. There is little change in the appearance of the cercariae free in the haemocoel. The penetration glands are less distinct and it is believed that they are operative in the escape of the cercariae from the alimentary tract of the second intermediate host and possibly in migration through the tissues surrounding the haemocoel as well. The tail is absent at this time, though just where decaudation occurs has not been established. It probably takes place during the penetration of the digestive tract of the second intermediate host, since the process has been completed prior to encystment of the cercariae.

The metacercariae appear as transparent, oval cysts with about the same refractive index as the tissues of the host and they are embedded in the larger muscles which extend from the body wall to the appendages. The cyst wall is very tough and attempts to digest it with N/10 HCl + 1% pepsin, and with 1% pancreatin were ineffective. The metacercariae are active within the cysts and the glands surrounding the excretory vesicle are distended, indicating that they are functional. It is suggested that they may be liberating their secretions posteriorly, either to aid in the formation of the cyst walls, or to provide a liquid medium for the metacercariae within the cysts. Goodchild (1943) described encystment in the cercaria of *Phyllodistomum solidum* (= *Cercaria conica*), a form with a similar glandular arrangement, which liberated its cystogenous fluid through the excretory pore.

Description of Stages in the Life History

The Adult

The mature worm, *Conspicuum icteridorum,* is found attached, principally by means of the powerful ventral sucker, to the lining of the gall bladder of the definitive host, *Quiscalus quiscula quiscula.* The adults are lanceolate in shape and range in length from 3 mm. to 6 mm., the average being 5 mm. (Fig. 10). For a detailed

description of the adult, reference should be made to the original account by Denton and Byrd (1951).

The Egg and Miracidium

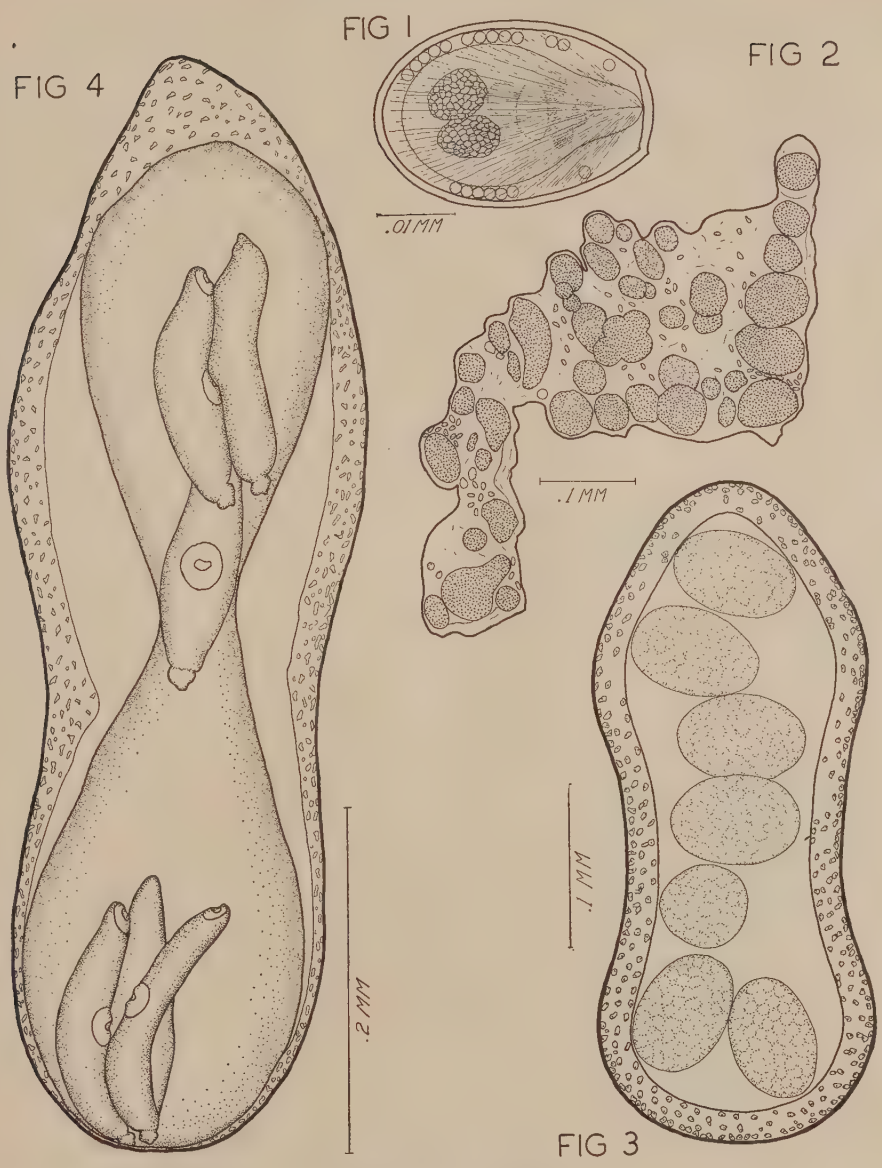
The mature egg, (Fig. 1), is operculate, 19–24 microns wide by 27–33 microns long, is yellowish to brown in color, fully embryonated when shed and the shell is so thick and opaque that it is difficult to observe the details of the miracidium. Between the shell and the miracidium there is a layer of spherical, refractile droplets. The miracidium is pyriform and is oriented with the smaller, anterior end close to or against the operculum. Movements of the miracidium within the egg were rarely seen and efforts to hatch the eggs were ineffective. The entire miracidium appears to be ciliated, with longer cilia toward the pointed end where they so obscure the stylet as to make its identity uncertain. Two large oval vesicles composed of irregularly shaped granules are plainly visible in the posterior part of the miracidium where they are oppositely situated. Their function is unknown but it is thought that they may have an excretory significance. A somewhat conical mass of very fine granules lies in the anterior part of the body and within the apex of this mass the base of the stylet is buried. When observed under oil immersion the granules are seen to be in a state of Brownian movement. When freed under pressure, the globules from the egg disperse in the Ringer's solution, the stylet becomes identifiable, and the large posterior vesicles maintain their identity. Under these conditions the miracidium was seen to move convulsively for a few moments but no locomotion was observed.

The Primary Sporocyst

The mature primary sporocyst is so extensive and so integrated with the softer tissues and with the tunica propria of the snail that it is difficult to determine its exact limits and it is impossible to remove the sporocyst intact. Under higher magnification the sporocyst seems to be covered with a thin epithelium and to be, in essence, an amorphous, saccate, non-branched structure, the body of which is filled with germinal cells and secondary sporocysts in various stages of development (Fig. 2). Slight pressure on the mature mother sporocyst causes rupturing and the release of daughter sporocysts and since at the time of this observation (70 days after infection), many of the daughter sporocysts already filled the spaces between the organs of the mollusk, it seems safe to consider them to have been in the migrating stage for at least some days prior to this. The secondary sporocysts apparently arise as small germinal masses within the primary sporocyst. The non-germinal tissue of the primary sporocyst is composed in large part of small irregularly shaped, rather widely spaced, mesenchyme cells between which run fine wavy fibers or striae of undetermined nature. No movement was evidenced by the primary sporocyst.

The Secondary or Daughter Sporocyst

Immature daughter sporocysts at the migrating stage of development are spherical to elongate structures of various sizes and manifest considerable motility (Fig. 3). One end is more pointed and more active, precedes in locomotion and might be designated anterior although no birth canal or pore is present and there is no gradient of development of cercariae toward that end. The walls of the sporo-



EXPLANATION OF FIGURES

PLATE I

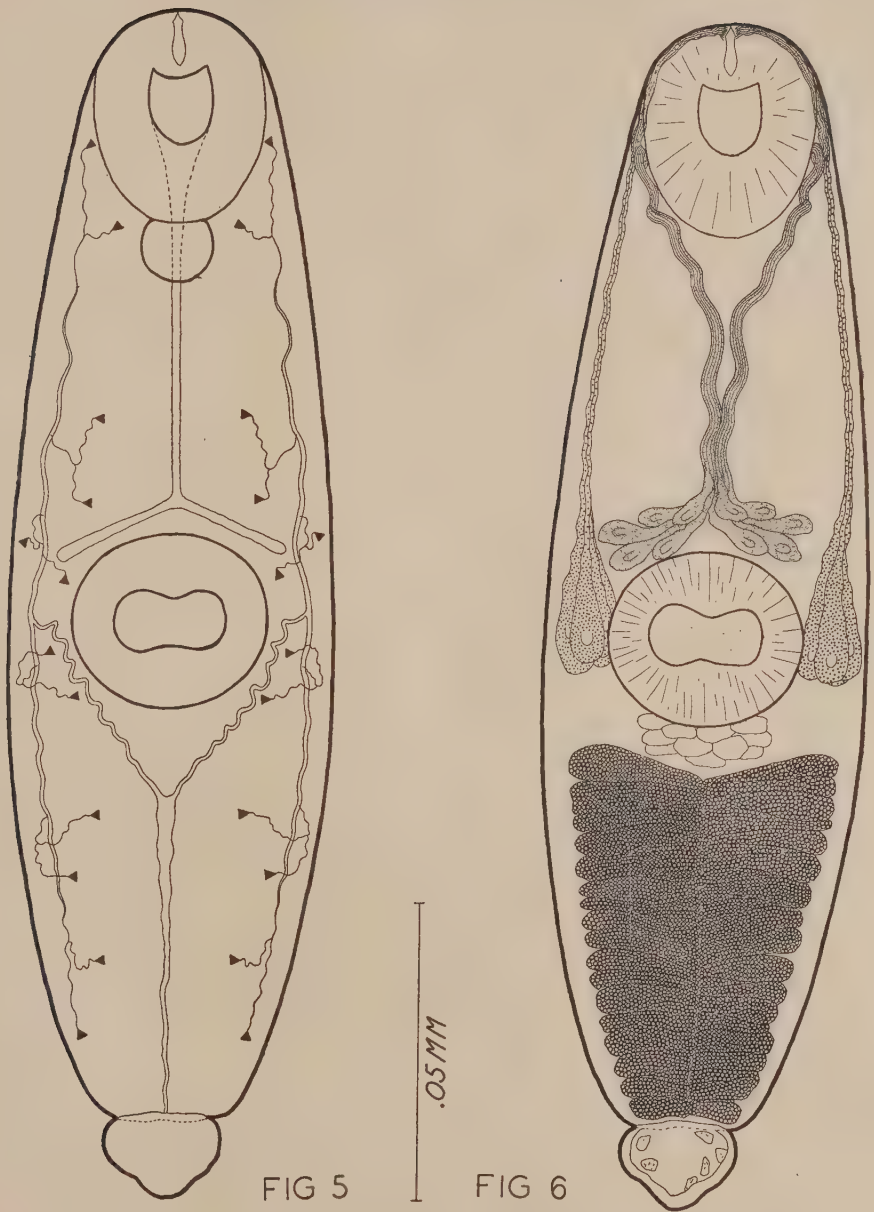
FIG. 1. Mature egg, showing details of miracidium.
FIG. 2. Piece of primary sporocyst, showing germ balls and developing secondary sporocysts.
FIG. 3. Immature secondary sporocyst, showing developing cercariae.
FIG. 4. Mature secondary sporocyst, fully extended and showing mature cercariae.

cysts are transparent and developing cercariae are clearly visible. A distinct excretory system is present in the thick wall of the sporocyst with the flame cells grouped somewhat toward the anterior end of the sporocyst.

Mature daughter sporocysts (Fig. 4) differ little in general appearance from those of the migrating stage. They measure 225–275 microns wide by 600–750 microns long and are first shed, at room temperature, 95–100 days after the snails are infected. In the mature sporocyst both ends are equally blunted and no excretory system was detected. A thick outer somatic layer surrounds a relatively thin, tough, acellular brood sac, endosac, or endocyst (Denton, 1944), within which the cercariae develop simultaneously. The somatic layer varies locally in thickness from 4–12 microns and appears to be somewhat syncytial in nature with nuclei irregular in shape and widely spaced and surrounded by fluid. The inner part, or endosac, of the sporocyst is in the form of a tube rounded and somewhat enlarged at the ends and constricted in the middle and containing 5–14 (average 7) brevicaudate cercariae. These larvae are surrounded by, or suspended in, a viscous, acellular fluid which fills the entire endosac and which Denton (1944), identified with the contents of the large posterior glands of the cercariae of *E. procyonis,* a similar cercaria, and which Maldonado (1946) attributed to the anterior glands of the cercariae of the dicrocoeliid, *P. fastosum.*

The Cercaria

The cercaria (Figs. 5, 6) is small, muscular, semitransparent, and aspinose, widest at the level of the acetabulum and equipped with a stylet and a short, somewhat pyriform tail. Sensory papillae are scattered over the surface of the body though more concentrated at the anterior end. In measurements taken on 25 hot-formalin-killed specimens, the length was 170–205 microns (average 186 microns) and the width was 46–54 microns (average 50 microns) at the widest part of the body. The oral sucker is muscular, subterminally situated with the opening directed anteriorly, and in a pocket in its antero-dorsal wall bears a small stylet only the tip of which is free. The oral sucker measures 35–40 microns long, by 27–32 microns wide, the average length and width being 37 and 29 microns respectively. The stylet measures 3–5 microns wide by 14–18 microns long. There is no prepharynx and the almost globular pharynx lies against the posterior border of the oral sucker and averages 12 microns in diameter. Extending from the pharynx is a very thin, thread-like oesophagus which continues posteriorly until it bifurcates a short distance anterior to the ventral sucker; the two short ceca continue mediolaterally to about the level of the equator of the acetabulum. The ventral sucker is situated a short distance posterior to the middle of the longitudinal axis of the body, the distance from its center to the anterior tip of the body being 88–103 microns and from the center to the posterior tip being 80–84 microns. The acetabulum is a deep, muscular, cup-shaped structure averaging 33 microns along the transverse axis and 30 microns along the longitudinal axis. Lying in a cluster just anterior to the acetabulum are ten penetration glands. These are flask-shaped structures filled with fine globules which give the glands a granular appearance. The ducts of these glands leave as a single large bundle but soon split into two bundles of five ducts each which pass anteriorly on opposite sides of the median plane. These bundles follow a sinuous course turning laterally around the pharynx and



EXPLANATION OF FIGURES

PLATE II

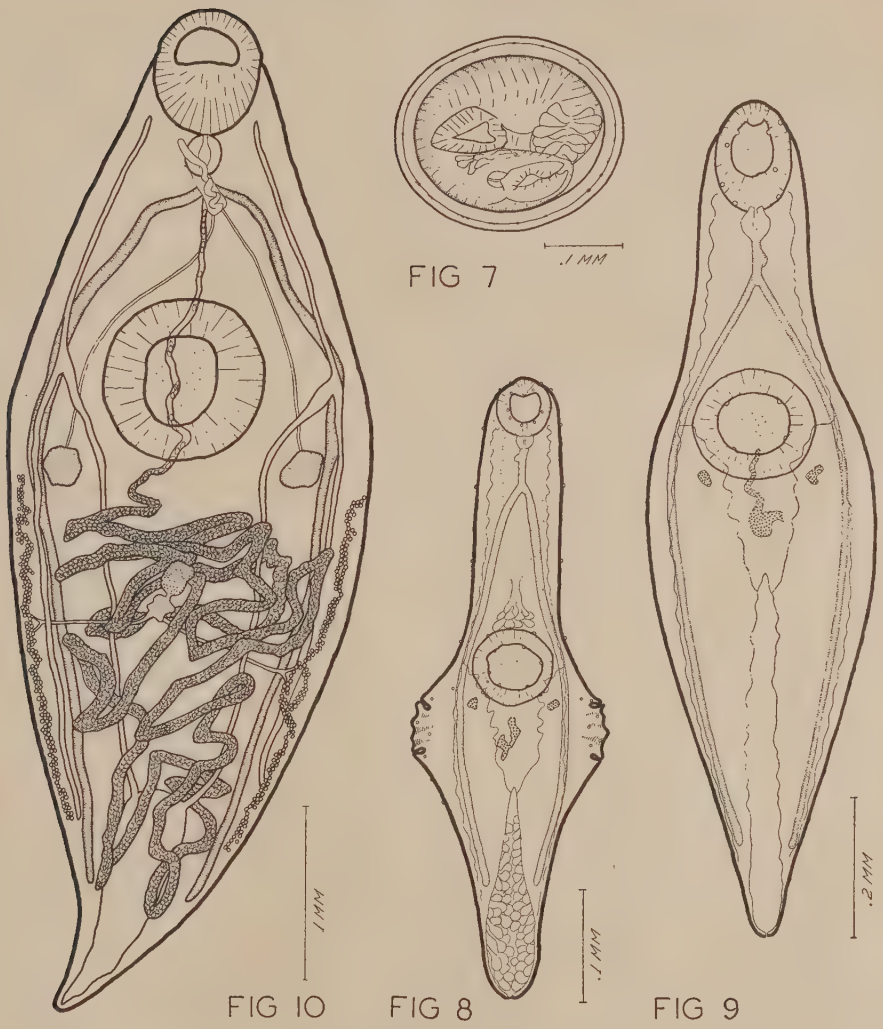
FIG. 5. Cercaria, showing details of excretory system.
FIG. 6. Cercaria, general morphology.

oral sucker then mediad to the pocket of the stylet where the ducts enter and apparently empty separately. On either side of the acetabulum are four or five glands so faint in outline as to be extremely difficult to locate and differentiate. Their ducts lie lateral to those of the previously described penetration glands and they proceed anteriorly in a much straighter course. However, they appear to enter the pocket of the stylet in conjunction with the ducts of the other glands. Just posterior and somewhat dorsal to the acetabulum lies the genital primordium, an indistinct group of round or oval cells only faintly visible in unstained specimens but rather heavily marked with Semichon's carmine. Posterior to the genital primordium the body is occupied by a number of large closely packed glands which are filled with globules and which surround and are attached to the excretory vesicle. The excretory vesicle extends from the terminal pore to near the posterior border of the germinal mass where a primary collecting tubule is given off on each side. These tubules proceed anterolaterally in a winding course to about the level of the equator of the acetabulum where each divides into an anterior and a posterior main collecting tubule. Each of these secondary tubules gives rise to three accessory collecting tubules, each of which bifurcates into two capillaries which terminate with flame cells. The flame cell pattern thus established for the cercaria of *C. icteridorum* is $2[(2+2+2)+(2+2+2)]$ (Fig. 5), and is identical with that of the adult stage (Denton and Byrd, 1951). The small knob-like tail is attached slightly subterminally, and appear to have adhesive qualities since the cercaria sometimes affixes itself to the substratum by means of this structure. The tail seems to have an internal space, and scattered about, particularly around the sides, are 7–12 oval to irregularly shaped cells with prominent nuclei. The average length of the tail is 16 microns and the average width is 19 microns.

The Metacercaria

No metacercariae were found in the terminal segments of the isopods, and the sizes of the cysts varied with their ages. The smallest cyst measured 116×148 microns and the largest 264×306 microns; the former represented a cyst probably only a few hours old and the latter one of approximately 75 days. An average measurement to be significant, therefore, would have to be determined on the age of the metacercariae. Through the transparent walls of the cysts the active metacercariae can be observed in considerable detail (Fig. 7). In young metacercariae the stylet is clearly visible, the tail is absent, and the glands surrounding the excretory vesicle proper are distended and no longer granular in appearance. In recently encysted metacercariae the cyst walls are thin, though tough, and are derived entirely from the parasite. As the cyst grows older an additional wall is laid down, presumably by action of the host, and this wall is inseparably applied to the original cyst wall. The stylet is extruded sometime during the first 20 days of metacercarial existence.

Metacercariae are teased from the cysts with difficulty and recently encysted specimens appear little different from the cercariae. At this stage the penetration glands have emptied and are indistinct; the germinal primordia and the papillae covering the body seem more clearly visible. There is no marked extension of the ceca and the overal growth of the metacercariae is slight. The posterior glands of the body are plainly separated so that there appears to be about fifty of them,



Explanation of Figures

Plate III

Fig. 7. Encysted metacercaria, infective stage.
Fig. 8. Infective metacercaria freed from cyst. About 70 days old.
Fig. 9. Young worm. About 12 days old.
Fig. 10. Adult worm.

though due to their overlapping arrangement it is difficult to get an accurate count. When released from the cyst the metacercariae are more active and seem stronger than were the cercariae.

Mature, or infective, metacercariae, when teased from the cyst, show much growth and differentiation (Fig. 8). Relaxed, living specimens of about 70 days age measured 175 to 238 microns at the widest point of the body and 443 to 675

microns in length, the average being 204 × 500 microns. In addition to overall growth, a certain amount of differential growth has taken place in the metacercaria. The body in the region between the level of the equator of the acetabulum and approximately the level of the midpoint of the long axis of the excretory bladder has grown laterally on either side, forming a thin, triangular-shaped, leaf-like protrusion which gives the metacercaria a shape reminiscent of the adult worm. The edges of these outgrowths are so thin as to be curled at times and well developed sensory papillae line their margins, creating the impression that the outgrowths have a sensory (tactile) function. Large sensory papillae are scattered about over the surface of the body but are most conspicuous on the ventral and oral suckers and on the anterior tip of the body, particularly in the region where the stylet was located and where now is situated a slight muscular protuberance which appears to be tactile in nature. The alimentary tract is now clearly marked, the ceca extending posteriorly to a level about midway of the excretory bladder. The excretory system is clearly delineated, the main vesicle being still gorged with the large gland cells. At this time, however, individual glands are often free in the lumen except for a single fine duct which serves as an attachment to the wall of the vesicle but permits shifting of the gland during movements of the metacercariae. As would be expected, the flame cell pattern is the same as that of the cercaria and the adult. The germinal primordia have now separated so that the testes and the ovary-complex are plainly visible in stained specimens and they appear to be in their definitive positions. Metacercariae freed at this age are extremely active in swimming movements and in exploratory and creeping activities. Their ability to extend themselves greatly is noticeable and this faculty is probably necessary for their successful movement through the biliary duct. The earliest age of infectivity is estimated at about 30 days.

The Young Worm

The youngest worms were obtained from the gall bladder 16 hours after the metacercariae were fed to the birds. They were identical in all significant features with the metacercariae. The only obvious change that had occurred was that the excretory vesicles had shed their glandular constituents.

Worms 10 days old had increased in length to 1.0–1.2 mm. and the lateral body outgrowths had smoothed out until the contours of the young worm were essentially that of the adult (Fig. 9). By 20 days the worms had attained a length of 1.9–2.2 mm. The worms continued to grow approximately at this rate until the adult size was attained. However, the full development of the vitellaria and the production of viable eggs did not occur until 2–4 weeks after the worms had apparently reached their maximum size (Fig. 10).

DISCUSSION

While the research here reported was in progress, Denton and Byrd (1951) described a new species of trematode, *Conspicuum icteridorum,* from the gall bladder of grackles. Since the adult worms discussed in the present paper agree in all major respects with the description of Denton and Byrd, they are assigned to *Conspicuum icteridorum* Denton and Byrd, 1951.

The unique position of *D. dendriticum* as the only dicrocoeliid trematode for which the complete life history was previously known, while at the same time it presents so simple and direct a cycle, makes its evaluation in terms of data on other

dicrocoeliid cycles difficult if not uncertain. The fact that no other of this group of trematodes has been reported to have cercariae directly infective to the definitive host makes it seem possible that here may be an aberrant case, perhaps even an example of an abbreviated life history parallel to that presented by Bacigalupo (1926, 1928) for the cestode (*Hymenolepis nana.* The cestode does not require a second intermediate host but may or may not use one. *D. dendriticum* does not require a second intermediate host but whether one can be utilized is not known.

The second most nearly complete cycle in the Dicrocoeliinae, that of *P. fastosum,* is of particular interest in that a reptile is involved either as the second intermediate host or as a transfer host. Maldonado (1945, 1946) discovered immature *P. fastosum* in lizards and obtained adult worms in cats by feeding them infected lizards. He did not, however, establish the manner by which the lizards became infected so that there is a strong possibility that another host is involved prior to the lizard, in which case the latter would serve only as a transfer host.

The importance and possible prevalence of transfer hosts in the life cycles of digenetic trematodes is well exemplified in the strigeid species, *Pharyngostomum cordatum* (Diesing) Ciurea, as reported by Wallace (1939). The adult parasite lives in the intestine of cats. The cercariae normally encyst in a variety of amphibians. The definitive host may acquire the infection by eating the amphibians or the metacercariae may be transferred without morphological changes through a series of hosts such as snakes, rats, chicks, and ducklings, any of which can infect the definitive host. The value of transfer hosts in the maintenance and dispersal of this species is obvious and it seems not impossible that the lizard is serving in a similar capacity for *P. fastosum.* The occurrence of the same species in a wide variety of hosts may also account for variations in different individuals and for the confusion in the recognition and identification of such specimens. Furthermore, the presence of similar parasites in unrelated hosts may suggest evidence of parasitic evolution. Stunkard (1947) suggested such an example in his discussion of the similarity between *Paradistomum* and *Platynosomum* and *Eurytrema.* He pointed out that morphological differences between these genera are slight and since species of *Paradistomum* are parasitic in the bile ducts of reptiles and since lizards serve as second intermediate hosts of *P. fastosum,* it is probable that the paradistomes are ancestral to the eurytremids which developed when reptiles were eaten by birds and mammals. Such an explanation would account for the bionomic and morphological resemblances between the species of *Paradistomum* and *Eurytrema.*

A comparison of various aspects of the morphological and developmental histories of those dicrocoeliid trematodes for which data are available indicates the existence of two divergent groups.

1) Those with long-tailed cercariae in which a relatively small number of daughter sporocysts are produced simultaneously, or nearly so, and in which cercariae are matured successively over a long period of time and escape by way of the birth canal in the sporocyst. Examples of this type are *D. dendriticum* and *B. americanum.*

2) Those with brevicaudate cercariae in which a large number of daughter sporocysts are developed continuously over a long period of time and in which the cercariae are matured simultaneously in each sporocyst and from which there is no active escape. Examples of this type are *C. procyonis, P. fastosum,* and *C. icteridorum.*

There seems to be no means, at present, of classifying the members of the above

groups on the basis of their host preferences. *D. dendriticum,* of the long-tailed group, is a mammalian parasite; so are *C. procyonis* and *P. fastosum* of the brevicaudate group. On the other hand, *B. americanum,* a long-tailed form, and *C. icteridorum,* a short-tailed species, are avian parasites. The idea of host specificity in the restrictive sense cannot always be applied, and here it seems there is considerable latitude in choice of hosts by closely related forms. When knowledge of the second intermediate hosts and the methods of infection are made known, it is likely that the significance of tail types to infection and to taxonomy will be resolved and the choice of hosts may be made clear.

A more detailed examination of the forms in question may serve to bring about a clearer concept of the relationship of the species and to further justify the proposal of the above groups.

D. dendriticum and *B. americanum,* as reported, have similar intramolluscan developments. The mother sporocysts are described (Denton, 1945), as being identical in structure and in that a small number of daughter sporocysts are produced over a short period or simultaneously. The morphology of the daughter sporocysts is similar including the presence of a birth canal. The cercariae are produced in a similar manner and their escape from the sporocysts is the same. The cercariae are alike in general body shape and in the possession of a well-developed tail. Both possess a stylet and large and small penetration glands which occupy similar positions in the body. The flame cell patterns are identical. They differ in that the cercariae of *D. dendriticum* are larger and possess more of the posterior penetration glands.

The intramolluscan development of *C. icteridorum* closely parallels that of *C. procyonis* as presented by Denton (1944), and of *P. fastosum* as described by Maldonado (1946). The mother sporocysts are similar in structure and in the production of daughter sporocysts over a long period of time. The daughter sporocysts are essentially identical in that they are closed structures bearing cercariae in an endosac, and are incapable of movement after being shed. The cercariae are matured simultaneously in all three species and are identical other than in a slight variation of size. This identity extends to the possession of a stylet, a rudimentary knob-like tail, the same flame cell pattern, and the possession and location of the anterior and lateral penetration glands.

Similar intramolluscan development of the two above groups as such, plus the similarity of the gross and detailed morphology of their intramolluscan stages, including the cercariae, make it appear logical that *D. dendriticum* and *B. americanum* are more closely related to each other than they are to *C. procyonis, P. fastosum,* and *C. icteridorum,* which appear to form a second closely related group.

SUMMARY

New evidence in the life cycle of an avian trematode of the subfamily Dicrocoeliinae is presented. An arthropod intermediate host is required.

The first intermediate host for *Conspicuum icteridorum* is established as *Zonitoides arboreus,* the intramolluscan development is traced, and the stages described.

The second intermediate host is established as *Oniscus asellus,* or *Armadillidium quadrifrons,* and development of the metacercariae within the second intermediate host is described. This is the first substantiated report of a second intermediate

host (in the true sense of the word) being involved in the life history of a trematode of the subfamily Dicrocoeliinae.

Metacercariae were fed to the definitive host, *Quiscalus q. quiscula,* and were recovered from the gall bladder 16 hours later. Worms matured in the definitive host approximately 12 weeks after the birds were infected.

The developmental cycle and morphology of the larval stages of *C. icteridorum* are compared with those of other members of the subfamily to which it belongs and for which information has been presented. The marked similarity in the intramolluscan development and morphology of larval stages of *Dicrocoelium dendriticum* and *Brachylecithum americanum* on the one hand, and of *Concinnum procyonis, Platynosomum fastosum* and *Conspicuum icteridorum* on the other, is pointed out.

The parasite here discussed is referred to the species *Conspicuum icteridorum* Denton and Byrd, 1951, and its position within the present taxonomic scheme is reviewed.

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RESEARCH NOTES

PRELIMINARY OBSERVATIONS ON A SEVERE EPIDEMIC OF MICROSPORIDIOSIS IN THE SMELT, *OSMERUS MORDAX* (MITCHELL)

During September and October of 1951 several hundred dead smelt, ranging in size from 1½ to 7½ inches were seen along the shores of Loon Pond, Gilmanton, N. H. In each of 134 autopsies the most striking feature was the greatly distended intestine covered with hundreds of white, glistening cysts. The cysts varied in size from 0.1 mm. to 2.5 mm. The majority of cysts in a given fish, however, were approximately the same size. The pyloric caeca were always heavily infected and occasionally a few cysts were found in the liver, heart and gonads. No cysts were found in the esophagus, stomach, spleen, kidneys, or body musculature. In all cases the intestine appeared to be the primary site of the infection; the lumen was partially, if not completely, occluded and little or no evidence of normal intestinal tissue could be found. The normal functioning of the intestine would seem to be completely impaired under these conditions.

An interesting side-note was the finding of one to two mature specimens of *Leptorhynchoides thecatus* in about 30% of the smelt. In all cases the worms found were dead, either free in the body cavity or lying on the external surface of the gut. This would appear to give further indication of the structural and functional disintegration of the host intestine.

Microscopical examination of the milky cyst fluid showed hundreds of pyriform spores measuring 3.5–5.4 micra in length, by 2–2.4 micra in width. Extrusion of the polar filament was accomplished by applying pressure on the cover glass. It ranged from 108–121 micra in length. Additional studies on the spores and developmental forms in stained smears and sections indicate that the parasite is *Glugea hertwigi* Weissenberg, 1911. Weissenberg first described *G. hertwigi* from the European smelt, *Osmerus eperlanus*. He found a 1–2% incidence in one-year-old fish. Schrader (Jr. Parasit. 7: 151; 1921) first noted its occurrence in the American smelt, *O. mordax*. He reported *G. hertwigi* from Lakes Sunapee (28%), and Massabesic (53%), N. H., and from the coastal waters of Maine (1.5–16%). It has been reported from *Fundulus heteroclitus* from Chesapeake Bay by Bond (Trans. Amer. Miscros. Soc. 57: 107; 1938), and by Fantham et al. (Parasit. 33: 186; 1941) in one of several smelt taken from Lake Edward, Quebec, Canada. The present paper, however, records the first instance in which this microsporidian infection has been observed in acute epidemic form.

Recently, the writer had the opportunity to examine 20 *O. mordax* seined from the brackish waters of the Oyster River, Durham, N. H.; 16 fish showed the characteristic picture of an infection with *G. hertwigi* although to a lesser degree than those from Loon Pond. This incidence is considerably higher than that reported by Schrader from smelt taken along the Maine coast.—A. James Haley, *University of New Hampshire, Durham, N. H.*

EIMERIA CYSTIS-FELLEAE VAR. *AMERICANA*, N. VAR. IN SOUTHERN CALIFORNIA LIZARDS

At least 61 species of *Eimeria* have been reported from cold-blooded terrestrial vertebrate hosts. Among the reptiles, the Ophidia appear to harbor about twice as many different species of *Eimeria* as are known from both Lacertilia and Chelonia together.

Although the great majority of *Eimeria* reported from reptilian hosts undergo their intracellular stages of development in the intestinal epithelium, a dozen species are known to parasitize epithelial tissue of the gallbladder and bile duct, with some even invading the liver, spleen and kidney tissue. Among several Southern California lizards examined, a coccidial infection of the gallbladder and bile duct was observed in eight out of 23 Island Night Lizards, *Xantusia r. riversiana*, from San Nicholas Island, and in one out of 12 side-blotched lizards, *Uta stansburiana*, from the Palmdale area in the Mojave Desert. The degree of host tissue invasion reached such severity in some cases that practically every epithelial cell of the biliary system showed parasites.

Debaisieux (1914; La Cellule 29: 433) described a similar coccidian species from a western European ophidian host, *Tropidonotus natrix*. The intracellular stages of *E. cystis-felleae* Debaisieux and of the Southern California species are practically identical, although in the case of the ophidian *Eimeria* no mention is made of an intracellular gametic association so common in the lacertid form. However, in the sporogonous cycle there is a slight difference. The sporozoites of *E. cystis-felleae* are described and figured as comma-shaped, possessing a large

vacuole with enclosed granules near one end and having cytoplasm which contains many inclusions. In contrast, the straight spindle-shaped sporozoites of the lacertilian species have a homogeneous cytoplasm and are devoid of any inclusions or vacuoles.

Since there are some minor dissimilarities between the two species of coccidia mentioned, and since there is a rather widely separated geographical distribution, as well as a taxonomic difference of hosts, the *Eimeria* observed in the Southern California saurian hosts should be considered as a new variety. The name *Eimeria cystis-felleae* var. *americana* is proposed.—YOST U. AMREIN, *University of California, Los Angeles, California.* Present Address: *Pomona College, Claremont, California*

NEW RECORDS OF ECTO AND ENDOPARASITES OF CHICKENS IN EGYPT WITH SPECIAL REFERENCE TO THE TAXONOMY OF *SUBULURA BRUMPTI*

Although it has long been recognized that poultry constitutes an important minor industry in Egypt, thus far there has been little systematic study of the parasites which infest fowls in this country. In one thousand two hundred fowls of native breeds collected from the area near Cairo, ten species of helminth parasites and five of external parasites were found. The helminths consisted of two trematodes; *Echinostoma revolutum* (Frölich, 1802), *Echinoparyphium recurvatum* (v. Linstow, 1873); four cestodes; *Raillietina* (R.) *echinobothrida* (Megnin, 1881), *Raillietina* (*R.*) *tetragona* (Molin, 1858), *Cotugnia digonopora* (Pasquale, 1890) and *Choanotaenia infundibulum* (Bloch, 1779); four nematodes; *Ascaridia galli* (Schrank, 1788), *Heterakis gallinae* (Gmelin, 1790), *Subulura brumpti* (Lopez-Neyra, 1922), *Acuaria* (*Dispharynx*) *spiralis* (Molin, 1858). The external parasites were the soft tick *Argas persicus* (Oken, 1818) and four species of lice; *Menopon gallinae* (Linné, 1758), *Menopon straminium* (Nitzsch), *Lipeurus heterographus* (Nitzsch, 1866), *Goniocotes hologaster* (Nitzsch, 1838).

During the systematic study of these parasites, it was noted that the generic position of the genus *Subulura* presented some difficulty and its correct position is still open to discussion, especially after the discovery of the life cycle of *Subulura brumpti* by Alicata (1939; J. Parasit. vol. 25, pp. 179–180). Yorke and Maplestone (1926; Nematode parasites of vertebrates, London) on morphological characters separated the genus *Subulura* from the family HETERAKIDAE (Railliet and Henry, 1914) and erected the new family SUBULURIDAE which included the genus *Subulura* (Molin, 1860) in the subfamily SUBULURINAE Travassos, 1914. This new family was established because of the absence in its members of the three large lips, characteristics of HETERAKIDAE, the presence of a definite cylindrical vestibule armed with three teeth at its base and the more feeble development of its precloacal sucker which is neither circular nor surrounded by a chitinoid rim. Both the families SUBULURIDAE and HETERAKIDAE were placed in the Superfamily OXYUROIDEA (Railliet, 1916). Chitwood B. G. and Chitwood M. B. (1950; An Introduction to Nematology. Baltimore) retained the previous taxonomic arrangement of Railliet and Henry by placing under the Superfamily ASCAROIDEA Railliet and Henry, 1915 the family HETERAKIDAE containing the subfamily SUBULURINAE Travassos, 1914 that contains the genus *Subulura*. Hyman (1951; The Invertebrates. Vol. III, p. 321, McGraw-Hill, New York) followed the arrangement of Yorke and Maplestone (1926).

The discovery of Alicata (1939) that arthropods act as intermediate hosts of *Subulura brumpti* would seem to relate this group with the superfamily SPIRUROIDEA Railliet and Henry, 1915 although it differs from this group on morphological grounds. *Subulura brumpti* differs from typical members of the ASCAROIDEA in the presence of a prominent posterior bulb in the esophagus and the use of an arthropod intermediate host. In case of the superfamily OXYUROIDEA, all its members are monoxenous. The only feature the heteroxenous *Subulura* has in common with monoxenous members of this superfamily is the bulbed esophagus. The genus *Subulura* is not a typical member of the superfamily STRONGYLOIDEA Weinland, 1858 in that a caudal bursa is missing, it has a three-lipped mouth and a prominent posterior esophageal bulb, and it disagrees in being heteroxenous. However, the intermediate host is an arthropod, while some reported life cycles of STRONGYLOIDEA have earthworms or snails as intermediate hosts.

The genus *Subulura* does not fit in any of the above mentioned superfamilies. If further studies of the life cycle of this group reveal that the intermediate host is invariably an arthropod, it may be necessary to erect a new superfamily to contain this and allied genera.—M. A. M. FAHMY, *Faculty of Veterinary Medicine, Fouad 1st University, Giza, Egypt.*

THE INCIDENCE OF *ENTEROBIUS VERMICULARIS* IN A METROPOLITAN SAN FRANCISCO PRE-SCHOOL NURSERY POPULATION

The nursery population, drawn from a group of working mothers, was limited to 30 pupils in each of 3 age levels (2, 3 and 4 years). Parents were provided with 6 test tubes containing modified NIH swabs (scotch tape, sticky side out, on the end of a glass rod). Written

instructions urged the mothers to sample the child on rising each day for six consecutive days. It took some parents 2½ months to complete the series; others never finished. Eight children provided no samples, 2 provided 3 each and 4 supplied 1 each. Seventy-six of the 90 children submitted either (a) 4 or more specimens or (b) a positive sample in less than 4 specimens. Forty-four (58%) of these 76 pupils were infected. The percentage of children infected in each of the 2, 3 and 4 year groups was 55, 65 and 54 respectively.

By comparison, Jacobs (1942, J. Pediat. 21: 497) found 75 infected children (33%) in a Stanford Lane Hospital (San Francisco) group of 228 young outpatients. Most of Jacobs' patients were available for only one sampling and being outpatients were probably tested after the morning toilet. Both of these factors would cause a survey to reveal an incidence of infection lower than the reality.—HERMAN ZAIMAN, WILLIAM LEEDY AND PATRICIA HOWARD, *San Francisco State College.*

A NEW HOST AND DISTRIBUTION RECORD FOR THE NEMATODE PARASITE, *SKRJABINGYLUS NASICOLA* (LEUCKART 1842)

During the month of December, 1950, of seven long-tail weasels (*Mustela frenata effera*) trapped within a ten-mile radius of Tollgate, Umatilla County, Oregon, two males and two females were infected with *Skrjabingylus nasicola.* A total of thirty-three nematodes were taken from the frontal sinus region. Fourteen of the nematodes were removed from one of the males and thirteen were removed from one of the females. *Skrjabingylus nasicola* was also recovered from the skull of a preserved specimen of *Mustela frenata effera* collected during the winter of 1946 in the vicinity of Tollgate, Oregon.

According to Swales (1938, Livro Jub. Travassos: 455–458) the first report of the geographic and host distribution of *Skrjabingylus nasicola* appeared in European literature as far back as the year 1868 (Weijenbergh). Baer (1931, Rev. Suisse Zoo. 38: 315–319) described this nematode as occurring in the frontal sinuses of *Putorius putorius* L., *Martes foina* Erx., *Martes martes* L., *Arctogale nivalis* L., *Arctogale erminae* L., *Lutreola lutreola* L., and *Lutra lutra* L., and mentioned the known distribution in Germany, Russia, and Switzerland. Baer failed to mention the form described by Cameron (1927, Jour. Helm. 5: 1–24) which established the distribution in Great Britian. However, Baer did refer to a possible record for North America in the nematodes described by Woodworth in 1897 from the frontal sinuses of *Mephitis* spp. and *Spilogale* spp. Cowan (1941, Rep. Provincial Game Comm., British Columbia: 40–45) reported *S. nasicola* in British Columbia from the short-tail weasel (*Mustela erminea cicognanii.*) Goble and Cook (1942, J. Parasitol. 28: 451–455) from New York State reported *Skrjabingylus nasicola* from mink (*Mustela v. vison*) and the New York weasel *Mustela frenata noveboracensis*). Sealander (1943, J. Parasitol. 29: 361–362) reported *S. nasicola* from mink in Southern Michigan.

Finding this species in *Mustela frenata effera* represents a new distribution record and infestation of the nematode in a new race of the long-tailed weasel. Grateful acknowledgment is due Dr. Thorkil Jensen, Wild Life Parasitology Laboratory at the University of Minnesota, for verification of the identity of *Skrjabingylus nasicola.*—RICHARD L. CLAPP, *Walla Walla College, College Place, Washington.*

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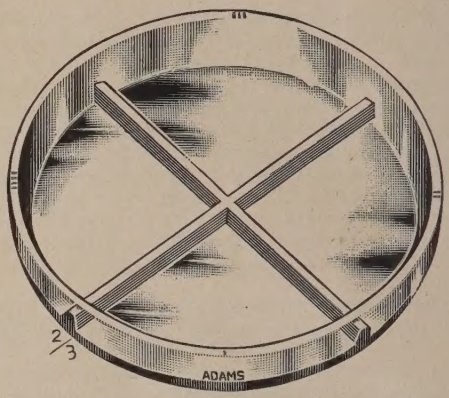

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